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THE RADIOGRAPHIC EVIDENCE OF THE INFLUENCE OF COD-LIVER OIL IN RICKETS

By E. A. Park and John Howland

(From the Harriet Lane Home and from the Department of Pediatrics, The Johns Hopkins University)

Cod-liver oil has been employed in medicine for so many years that it is difficult to say when it was actually introduced. Apparently it has been a folk remedy for centuries. During the last hundred years it has been used more or less extensively, particularly with children, for tuberculosis and for almost all diseases associated with wasting. It has also been employed in rickets.

Pharmacological evidence for the beneficial effects of cod-liver oil is slight. According to Cushny[1] it apparently owes its favorable influence chiefly to the fact that it is an easily assimilable form of fat. Meyer and Gottlieb,[2] however, indicate that there may be some virtue in cod-liver oil other than its ready emulsification and easy assimilability, inasmuch as they say that these properties "are not sufficient to explain its other real or fancied curative properties."

Although cod-liver oil has been used very largely in the treatment of rickets, there has not been complete unanimity of opinion regarding its curative properties. Some have regarded it as most effective. Perhaps it is fair to say that the majority have looked upon cod-liver oil as a substance which was of some value in rickets but in no way to be considered as a specific. To this latter class we confess that we ourselves have belonged.

Metabolism experiments, especially those of Schabad, have seemed to show definitely that cod-liver oil is of value. Schabad[3] found that cod-liver oil alone increased the retention of calcium in rickets, that sesame oil had no effect, that phosphorus alone had no effect, that cod-liver oil and phosphorus had a greater effect than cod-liver oil alone. These results have been essentially confirmed by Ernst Schloss.[4]

There are certain objections that can be raised against conclusions drawn from metabolic studies in almost any chronic disease. The results are determined during only a very small fraction of the total disease period and may not indicate satisfactorily what is going on during all of the time. Moreover, with calcium determinations it is a well-known fact that, owing to the large amount of calcium in the stools, erroneous conclusions may be reached, unless the stools for the period of analysis are sharply marked off; but the accomplishment of this is not always an easy matter. It seemed to us that direct ocular proof regarding the effect of cod-liver oil could be furnished by means of radiograms. We have not been able to find that the influence of cod-liver oil upon rickets has previously been determined in this way. Phemister[5] had used radiograms to follow the effect of the ingestion of phosphorus on different diseases involving bones. Since the appearance of the preliminary report of our work[6] he has published a paper upon the influence of phosphorus in rickets.[7] In a few instances the phosphorus was combined with cod-liver oil. He did not use cod-liver oil alone.

The experiments were planned to be as simple as possible. All the patients were in the hospital during the period of

observation, because our experience had taught us that the administration of cod-liver oil was too uncertain if left to parents at home. In so far as possible, and it was usually the case, the children were maintained upon the same diet as that upon which the rickets had developed. This was chiefly milk and cereal. The diagnosis of rickets was made by physical examination and by means of radiograms. As soon as the latter had been obtained, plain cod-liver oil was given in amounts varying from 2 to 4 c. c. three times a day. From time to time radiograms were taken. The rickets in all our cases was severe. In some it had existed for a long time without treatment.

What are the criteria upon which a diagnosis of rickets can be made from radiograms? They are to be found (a) in the shaft and (b) at the extremities of the long bones. With rickets there is a reduction, more or less, in the inorganic material of the bone. This is the substance which is impervious to the X-ray, and to the extent that this material is reduced, therefore, the shaft becomes less capable of casting a shadow. One of the most distinctive appearances of rickets is a reduction in the density of the shaft. In mild rickets the change is difficult to detect, in the more severe it is unmistakable. The cortex does not appear homogeneous and solid, but porous. As the trabeculæ which compose it have a parallel longitudinal arrangement, the cortex may appear lamellated. By the reduction of inorganic material the bones are weakened. Curvatures of the long bones are frequent and fractures much more common than would be supposed from clinical examination. The fractures are frequently subperiosteal without separation of the fragments. Callus formation is strikingly small in amount.

The configuration of the shaft of the bone differs very greatly according to the duration of the disease. In young, especially premature, infants the shafts are very thin and delicate and the cortex of linear thickness. In children of the second and third years the shafts are frequently increased in diameter and may have lost much of the normal form. The cortex is often of great thickness. This latter change is probably due to the fact that, in a conservative manner, nature attempts to compensate for the weakness of the shaft by a thickening of the periosteum. With the attempts at repair and the relapses that undoubtedly take place through months and perhaps years (for rickets is by no means necessarily a continuous process), more and more thickening of the periosteum results and more and more of the new material is by metaplasia transformed into bone.

In the normal bone the transition from the shaft to the cartilage is abrupt. Under all circumstances the end of the normal bone is sharply outlined. The earliest rachitic lesions at the junction of cartilage and shaft cannot be detected by means of the X-ray. As the process advances, a change is noticed at the cartilage-shaft junction as an indistinctness in the outline of the end of the shaft, which becomes progressively less clear cut. The shaft seems to terminate in thin, thread-like prolongations, which together give an appearance somewhat resembling a fringe. In the most advanced cases the rachitic "fringe" may be several millimeters long. In many children suffering from rickets cupping of the ends of certain of the long bones can be demonstrated. Just before its termination the shaft becomes broadened and at its line of junction with the cartilage forms a crater, often of considerable depth. When the end of the shaft has this concave appearance, it is usually possible to trace out the circumference of the crater. The cupping is much commoner at the ends of some bones than others. The lower end of radius and ulna and the upper end of the fibula are especially apt to be affected. In some of the most pronounced cases of rickets not only is no cupping present, but there is no enlargement at all toward the extremity of the bone. In these cases the shaft seems to terminate in an irregularly shaped stump. The reason for the peculiar appearance is that the new bone formed after the initiation of the rachitic process, free from lime salt deposit and therefore not capable of casting a shadow which distinguishes it from the surrounding soft parts, is very extensive. The shaft does not end where it ceases to be visible with the X-ray. It is prolonged onwards in the direction of the epiphysis, but is of such a composition as to cast no distinguishable shadow. When the rachitic process is as extensive as here described, there seems to be an abnormal distance separating the ends of the shafts at the joints, as, for example, the femur and tibia at the knee. If the rachitic process at the junction of cartilage and shaft is extensive, the center of ossification may not be visible at all, or it may appear much smaller than it should be to accord with the age of the subject, or its outline may be extremely faint. The epiphysis may be dislocated or at least bent over to one side. The changes at the junction of cartilage and shaft are much more marked at the ends of some of the long bones of the extremities than of others, but in advanced rickets changes at the junction of cartilage and shaft can be seen in all the long bones.

After treatment with cod-liver oil changes are detected by radiogram about the end of the third or fourth week, occasionally a little before. It is true that the deposition of salts must reach a certain magnitude before the deposits are detectible by so coarse a method as the X-ray. We have, nevertheless, some information in addition to that afforded by the X-ray to show that salts are not deposited immediately. One child died of an intercurrent disease six days after and another twelve days after the beginning of cod-liver oil medication. In the bones of neither of these children was there any deposition of calcium salts appreciable on microscopical examination. It is probable that in some children, particularly those in whom the disease is just entering upon a stage of repair, the deposition of lime salts occurs more readily than in those who are in the midst of an active and advancing rickets. The first evidence of repair is usually in the form of a line, always broken, beyond the extremity of the shaft, between this and the center of ossification, which latter may or may not be visible. A relatively clear space is often left between this line and the end of the bone. As time advances, this new line becomes thicker and thicker, and more dense than other parts of the

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

FIG. 5.

FIG. 6.

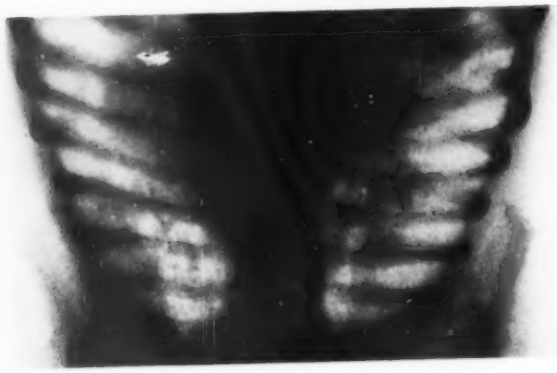

FIG. 7.

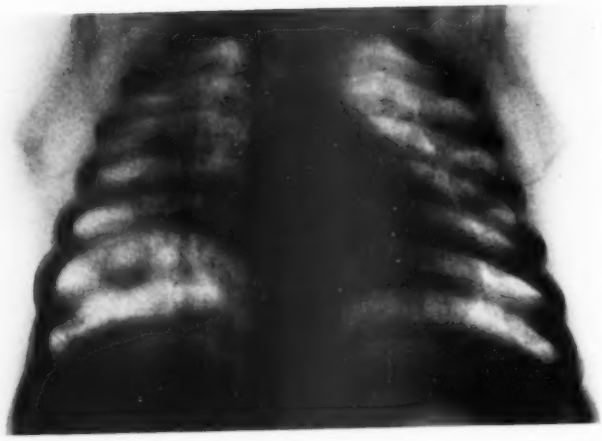

FIG. 8.

FIG. 9.

FIG. 10.

FIG. 11.

FIG. 12.

FIG. 13.

FIG. 14.

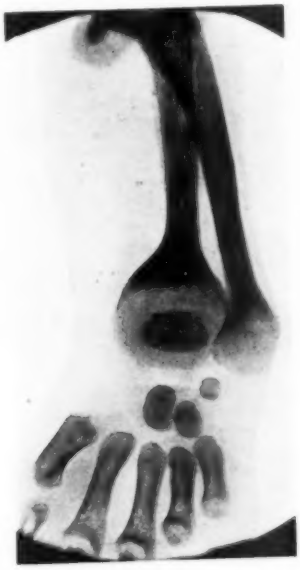

FIG. 15.

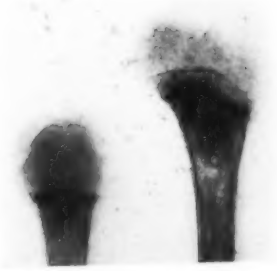

FIG. 16.

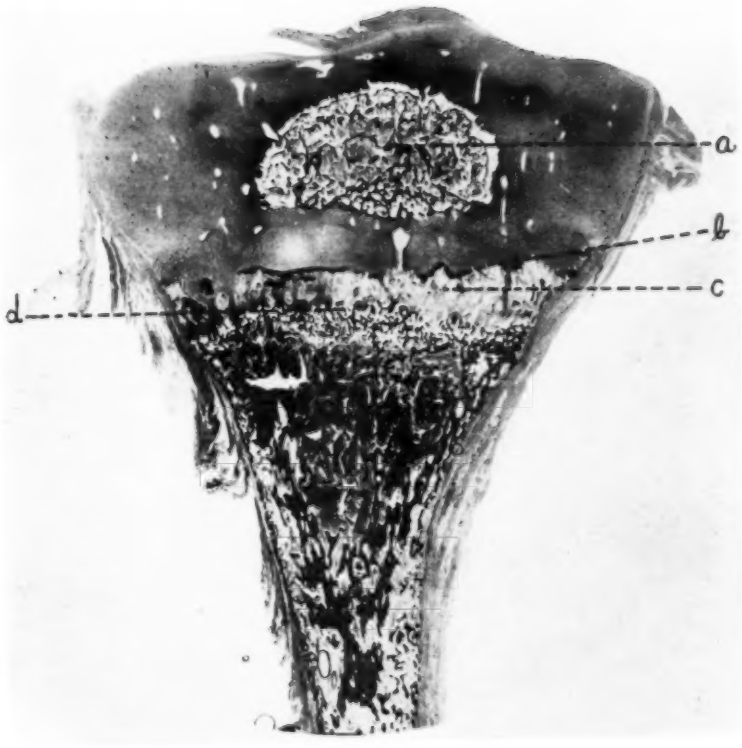

FIG. 17.

FIG. 18.

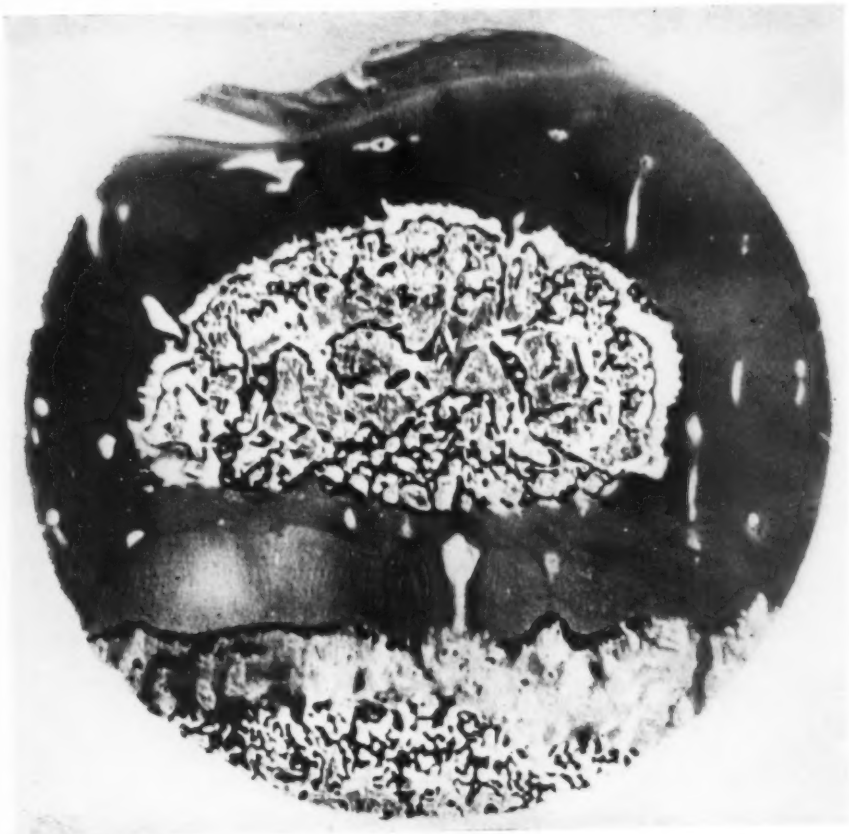

FIG. 19.

bone. Gradually the clear area between this line and the end of the shaft becomes filled in, until it can no longer be appreciated. At times there is not this linear formation, but the whole extremity of the bone which has before been invisible gradually comes into view. Pathological studies, chiefly by Schmorl,[8] have shown that in the process of healing the deposition of calcium salts takes place first in the cartilage on the epiphyseal side of the transitional zone, that is, in the place where the lime salts would have been deposited provided there had been no rickets. The deposition in this situation is the cause for the linear shadow in the radiogram. Gradually, the material in the transitional zone is transformed into bone and becomes infiltrated with lime. The repair of the transitional zone is time-consuming. When the rachitic process has been of such duration and is so extensive that the shaft seems to terminate in a stump, under cod-liver oil administration the true end of the bone gradually becomes visible. The deposition of lime salts frequently takes place in such a manner as greatly to increase the crater-like appearance. The end of the shaft seems like a cup or socket embracing the center of ossification. At the end of two or three months calcification of the ends of the bones seems to be complete. The finer architecture of that portion of the shaft which was the seat of the rachitic process is not, however, normal. The normal arrangement of the trabeculæ has not been restored.

As the result of the deposition of lime salts in the transitional zone the long bones seem to have grown greatly in length in a very short time. They probably *have* grown somewhat under the influence of the cod-liver oil. Very little of the apparent increase in length is, however, real. The portions that have been unable to cast shadows before are now plainly visible in the radiogram. The potential has been transformed into actual lime-containing bone.

Swelling of the costochondral junctions which may not have been demonstrable by radiogram before the administration of cod-liver oil can readily be detected in many instances. The swellings appear like buttons on the surface of the chest. The administration of cod-liver oil does not cause the enlargement of the costochondral junctions to diminish rapidly in size. On the contrary, when infiltrated with lime salts in this way, they must become more and more firm for a considerable period of time.

Increase in the density of the shaft takes place coincidently with the changes at the extremities, but it is a more slowly developing process, so that the bone may still be distinctly less dense than normal at the end of three months, when the extremities are, so far as can be determined, entirely calcified. Periosteal thickening, whether previously demonstrable or not, becomes strikingly apparent as the result of cod-liver oil medication. In extreme cases even the trabecular arrangement of the thickened periosteum can be appreciated. In contrast to the longitudinal arrangement of the trabeculæ of the cortex the periosteal trabeculæ are frequently arranged so that they are perpendicular to the long axis of the bone. When there have been fractures, the evidence of callus formation is usually greatly increased. The centers of ossification, previously invisible or faint, become clearly outlined. They also increase in size.

In our studies which have comprised in all some 50 cases, the results have been uniformly consistent. We feel justified in saying very definitely that cod-liver oil brings about a change in the bones which, if the diet be not too faulty, amounts to complete cure. The change is not noticeable at once, but is readily demonstrable in almost all cases by the end of a month. In two or three months so much infiltration with salts has taken place that the extremities of the bones, except for deformities, are practically normal, and only differences in the finer architecture of the ends of the bones indicate the previous existence of a rachitic process. We look upon cod-liver oil as a specific for rickets. We have not seen it fail in any single instance and we have known it to cure the rickets even though the children were dying of some other disease. Thus, one child with a sarcoma lost the radiographic evidences of rickets, though succumbing to the malignant growth, and another child, who was hanging between life and death as the result of a severe thoracic involvement and who finally died of pneumonia one month after treatment with cod-liver oil, did not fail to show calcium deposition in the bones both by radiograms and by microscopical examination. We know of hardly another drug that in disease exerts so regular, certain and specific an effect as does cod-liver oil in rickets.

Our thanks are especially due to Miss Mary S. Smith of the X-ray Department of the Johns Hopkins Hospital for her constant interest and cooperation in procuring the radiograms.

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DESCRIPTION OF PLATES

FIG. 1.—A. T., aged 13 mos., colored. Before treatment.

FIG. 2.—A. T. After treatment with cod-liver oil for 1 mo. 24 days. The actual terminations of lower ends of radius, ulna and humerus, previously invisible, have become visible. Apparent dislocation of epiphysis of radius. Marked periosteal thickening. Centers for carpal bones larger and more distinct.

FIG. 3.—A. T. Before treatment. Shafts of tibia and femur terminate abnormally. No enlargement towards epiphysis. Appearance as if ends of shafts had been sheared off. Femur is fractured. Incomplete fracture of tibia. Cortex of tibia has characteristic porous appearance. Periosteum does not appear thickened.

FIG. 4.—A. T. After treatment for 1 mo., 24 days with cod-liver oil. The fan-shaped lower ends of femur and tibia have come into

view. The old blunt terminations of the shafts are still visible. Great thickening of the periosteum of tibia, fibula and femur. Nuclei of ossification greatly increased in size. Apparent growth of bones in length.

Fig. 5.—H. G., aged 10 mos., colored. Before treatment. Great rarefaction of all bones. Nucleus of lower epiphysis of humerus and radius invisible. Slight evidence of periosteal thickening.

Fig. 6.—H. G. After treatment with cod-liver oil for 42 days. Apparent increase in length of radius and ulna. Deposit of salts in previously uncalcified portions of shaft renders these portions capable of recognition. Dark, irregular line at lower ends of shafts of radius and ulna caused by dense deposit of salts in portion of cartilage bordering on transitional zone. Transitional zone itself still incompletely calcified. Architecture of this zone different from that of shaft. Centers of ossification of lower epiphyses of radius and humerus visible. Extreme degree of periosteal thickening now evident. Bones much more dense.

Fig. 7.—H. G. Before treatment with cod-liver oil. Costochondral junctions cannot be made out.

Fig. 8.—H. G. After treatment with cod-liver oil for 42 days. Costochondral junctions now plainly visible. On the right side they appear as rings.

Fig. 9.—H. M., aged 11 mos., colored. Before treatment with cod-liver oil. Carpal bones invisible. Note distance from ends of radius and ulna to metacarpal bones.

Fig. 10.—H. M., after treatment with cod-liver-oil for 28 days. Dark, irregular, horizontal lines at extreme ends of radius and ulna mark calcium deposits in zone of cartilage on distal side of transitional zone. Transitional zone, still uncalcified, pale in contrast. Periosteal thickening striking. Two centers of ossification for bones of carpus visible. With apparent lengthening of radius and ulna, distance between them and metacarpal bones is noticeably diminished. Metacarpal bones share in the changes.

Fig. 11.—H. M., after treatment with cod-liver oil for 1 mo. 24 days. Density of all bones greatly increased. Calcification of lower ends of radius and ulna probably complete but disarrangement of finer structure of bone still remains.

Fig. 12.—J. J., aged 10 mos., colored. Before treatment.

Fig. 13.—J. J., after treatment with cod-liver oil for 1 mo. A faint, broken line caused by a deposit of lime salts marks the junction of cartilage and transitional zone. True length of shafts of bones now appreciable. Changes extremely early.

Fig. 14.—M. C., aged 21 mos., colored. Before treatment with cod-liver oil. True ends of shafts of radius and ulna not visible. Both bones look as if cut off.

Fig. 15.—M. C. After treatment with cod-liver oil at home intermittently for six or more months. (Case does not belong to hospital series.) Both radius and ulna now terminate in huge bulbous enlargements. That of radius seems almost to envelop large nucleus of ossification.

Fig. 16.—J. J. Upper ends of femur and humerus freed from soft tissue. Deposits of lime salts in cartilage on epiphyseal side of rachitic transitional zone are clearly visible. Deposits are fragmentary and hence the line is irregular. Characteristic porosity of cortex.

Fig. 17.—J. J. Section through upper end of tibia 200 microns thick. Stained with H. and E. and silver nitrate. Section cut slightly on the bias.

(a) Nucleus of ossification incompletely rimmed round with freshly deposited line of calcium phosphate.

(b) Broken line of calcium phosphate deposition at junction of cartilage and transitional zone.

(c) Transitional zone composed of cartilage, osteoid, connective tissue, blood vessels and marrow. No one of these elements casts a shadow distinguishable from those cast by the soft tissues. This zone, except at periphery, still free from calcium.

(d) Termination of that portion of shaft which was visible in radiogram.

Fig. 18.—J. J. Higher power magnification of calcium deposit in proliferative cartilage bordering on transitional zone.

Fig. 19.—J. J. Higher power magnification of nucleus of ossification. Enlargement of nucleus of ossification, seen in radiograph after treatment with cod-liver oil, due to formation of a fresh line of calcium phosphate deposit outside old.

LIPODYSTROPHIA PROGRESSIVA*

WITH REPORT OF A CASE

By Henry Lee Smith, Baltimore

Lipodystrophia progressiva is the name given by Arthur Simons[1] of Berlin to an uncommon disorder, a case of which he reported in detail in 1911.

The affection is characterized by a symmetrical, slowly progressive and almost complete disappearance of the fat from the subcutaneous tissue of the head, face, neck and upper extremities, and also from the trunk as far as the pelvic bones and folds of the groin where the fat absorption abruptly ends. This peculiar form of wasting is first noticed in the face whence it creeps downward over the parts of the body mentioned.

All cases of lipodystrophia, however, do not conform to the description just given, for in a few instances—in the male almost exclusively—the emaciation remains limited to the face and neck, or to the face, neck and thorax, as is shown in the cases reported by Husler,[2] Hertz and Johnson,[3] Batty Shaw,[4] Weber[5] and others. Likewise in females, there may be variations as to the parts of the body showing fat atrophy. In Lewandowsky's[6] case, for example, the face and trunk were atrophied but not the arms. In cases reported by Barraquer,[7] Boissonnas[8] and Herrman[9] there was no loss of fat in the abdominal walls, and in one case, that reported by Laignel-Levastine and Viard,[10] the face, apparently, was unaffected.

The term lipodystrophia progressiva is not altogether an appropriate one, for the buttocks and lower extremities do not waste, but on the contrary show an excess in the accumulation of subcutaneous fat, such as is seen in many women at middle-age. This fat increase in the lower parts of the body, however, occurs almost wholly in the female. It is symmetrical in distribution, invading first the buttocks and then, successively, the thighs and legs. Exceptionally, there may be a decided asymmetry in the contour of corresponding parts, as is well shown in the illustrations of Laignel-Levastine and Viard's[10] case. The feet and ankles are not implicated.

* This article was read in abstract, and the patient exhibited before the Baltimore Medical Society, Nov. 5, 1920.

In the 26 characteristic cases of progressive lipodystrophy found in the literature, *i. e.*, the cases showing both the atrophic and hypertrophic features of the disease, only two of the patients were males, a boy six years of age, whose case was reported by Boissonnas,[8] and Gerstmann's[11] patient, a soldier 32 years of age. Both of these reports were published in 1916, and until that time most of the authors had seemed inclined to regard the predominance of the gluteofemoral fat as belonging to the female sex. Spear[12] ventures the opinion, that "this characteristic is accentuated by the efforts made to over-nourish the patient to counteract the wasting which takes place in the upper portion of the trunk, face, etc." It is well known that excessive fat of the buttocks, "steatopygia," is seen as a normal development in the female of certain African races.

The increase in the size of the buttocks is usually not noticed until some years after the onset of the facial wasting. Feer[13] thinks it is possible that the fat atrophy and the fat hypertrophy begin simultaneously and progress concurrently, and that early evidence of gradual enlargement of the lower regions of the body might be obtained by accurate measurements. In Simons'[1] first case, the obesity of the lower parts of the body apparently had preceded by several years any wasting of the upper parts. In one case, that of Laignel-Levastine and Viard,[10] the legs first became enlarged, then the thighs and, finally, the buttocks.

The first case of the disease was published by Barraquer[7] of Barcelona in 1906 under the heading, "Histoire clinique d'un cas d'atrophie du tissu cellulo-adipeux," but as far back as 1895 Sir William Osler, when in America, had observed the characteristic features of this singular malady in a girl of 10 years of age. The wasting had begun in the face at the age of five years and had been followed by extreme emaciation of the trunk and upper extremities. It was ascertained in 1913, that the emaciation had remained practically at a standstill, but that the patient had enjoyed good health, and at the time of inquiry, felt well and was actually stronger than most women. Sir William Osler did not himself report the case, but gave Dr. F. Parkes Weber[5] permission to record it.

In 1907 Harry Campbell[14] exhibited a typical example of the disorder before the Clinical Society of London, which he described as a "Disappearance, more or less complete, of the subcutaneous fat above the region of the lower extremities." Pic and Gardère[28] reported, in 1909, "Un cas d'atrophie généralisée de la face et de la région sus-ombilicale du corps avec pseudohypertrophie de la région pelvienne et des membres inférieurs."

Simons[1] applied the term "lipodystrophia progressiva" to the case reported by him in 1911, and since then the disease has become known by that name. Gerhartz[26] thinks the epithet misleading. He preferred to call the case, published by him in 1916, "lipodystrophia progressiva superior." E. Feer[13] of Zurich, in an elaborate article which appeared in 1915, reported two cases, one of which had been referred to him by Boissonnas. The last publication that I have seen on the subject is a brief description of a case in a boy reported by Leipoldt,[25] of South Africa, in February, 1921.

The literature was reviewed by Weber[5] in 1917 and by Boissonnas[8] in 1919. Three Americans, Herrman,[9] Spear[12] and Epler[27] have reported one case each. About 27 well-defined cases have been published, and, additionally, about half as many of the limited or incomplete form. Of the former group, which is made up of 25 females and 2 males, the cases are presented more or less in detail and the diagnosis seems clear, but in the latter group, all of whom are males, the diagnosis of some of the cases is made on insufficient findings, and is therefore, to say the least, of questionable value.

The general appearance of a well-marked case of lipodystrophia progressiva in the female is grotesque. The eyes are usually set back. The malar arches stand out between the sunken temples and hollow cheeks. The face is deeply furrowed when the patient smiles. The neck is thin and stringy looking. The clavicles, scapulæ, and vertebral spines become prominent, and the intercostal spaces are plainly visible. The muscles beneath the lean skin are clearly outlined when made tense, giving the impression of over-development. The breasts are hard and pendulous. The thin abdomen and the conspicuously obese buttocks, thighs and legs complete the incongruous picture.

The onset is insidious. There is no fever or other constitutional disturbance of consequence. In the early stages of the atrophy, there may be vague aches and some malaise, and later a sensation of chilliness, hyperidrosis and slight nervousness.

Because of the emaciated condition, tuberculosis is generally suspected and the patients themselves naturally become apprehensive as to their condition and sensitive as to their appearance, but a definite causative relationship between progressive fat dystrophy and mental change is lacking. The functions of the cranial and peripheral nerves may be regarded as normal. Such clinical phenomena as exaggerated tendon reflexes, abolished unilateral plantar response, facial tic, diminished pupillary response, unilateral tremor and nystagmus, which have been recorded in isolated cases, are more probably due to factors other than those concerned with the causation of lipodystrophia. All investigators admit the absolute soundness of the muscular and osseous systems, and also, the normal muscle and nerve response to electric stimulation.

The digestive, respiratory, renal and cardiovascular systems are not abnormal. The blood picture proves negative.

The skin of the lean areas is natural in color, smooth, soft and freely movable over its subadjacent plane. It forms a thin fold when picked up, and when stretched, shows no loss of elasticity.* There is usually no disturbance of the cutaneous

* Had the cases so hastily reported as probable lipodystrophia in the male been more carefully examined, doubtless some of them might have been proven to be identical with that even rarer condition known as "elastic skin," examples of which are seen in the so-called "elastic-skin men" or "India-rubber men" of the circus side-shows. The cause of the extreme extensibility of "elastic skin" is not clear, and more or less confusion exists as to its histopathology, which only quite recently has been studied by Duhring, Kopp, Seifert, Du Mesnil and

secretions. Campbell [11] reported a foul smelling sebum in his case, and Feer [12] refers to a "peculiar smell" which persisted in his patient even after repeated bathing.

Examinations of pieces of skin and subcutaneous tissue removed from the emaciated areas in the living patient are recorded by Simons,[1] Christiansen,[10] Feer [12] and for my own case. The findings are uniformly the same. The epidermis and corium are normal, and the one histologic change is practically the entire absence of fat in the subcutaneous tissue. Simons,[1] in 1913, in a thorough study of his first patient, clearly demonstrated this lack of fat by an excellent illustration of a section of skin and subcutaneous tissue removed from the thorax, near the axilla, and, by way of contrast, he showed in another illustration the abundant presence of subcutaneous fat in a section from a piece of the scalp taken from a healthy, but not obese man, 34 years of age. He pointed out further that a piece of skin excised from the buttocks of his patient was macroscopically and microscopically normal. It is known that one to several millimeters of fat remain beneath the skin of greatly emaciated individuals suffering with such disease as tuberculosis, cancer and the like.

The various metabolism tests including the metabolic rate were found to be practically normal in Simons' case, but in my case the CO_2 output exceeded the normal by 36.8 per cent. In testing out the fat metabolism, Feer found no lipemia after his patient had been given a meal of 200 gm. of 20 per cent cream, and in Simons' first case there was no increase in the lipase of the blood.

Further investigations by Feer showed that a subcutaneous injection of pilocarpin produced only slight moistness of the cheeks, but that there was a copious outbreak of sweat on the chest, back, arms, forehead, belly and lower extremities. The pulse, respiration and intestinal peristalsis were uninfluenced. A subcutaneous injection of one milligram of adrenalin produced after three minutes a rise of 50 points in the blood pressure. No glycosuria followed the ingestion of 100 gm. of glucose.

There is no general enlargement of the lymph nodes. Moderate increase in size of the thyroid with evidence of more or less over-activity of this gland has been noted in a number of cases.

Two autopsies have been reported. The first was in the case of Husler's [3] patient, an undersized boy, who died in his 14th year from epidemic cerebro-spinal meningitis. At six years of age he showed signs of bifacial atrophy. The wasting, however, remained limited to the face. The muscular, osseous and nervous systems were normal. The autopsy was done mainly with reference to the meningeal condition. As regards the endocrine glands, the thymus was present, the genitalia were infantile, the suprarenals were found enlarged and contained a whitish medullary substance and a cortex rich in fat and pigment.

The second autopsy was reported by Weber and Gunewardene [20] in the case of a girl previously reported by them as showing a well-marked lipodystrophia progressiva. Death occurred at the age of 13 years from pyemia following an operation for mastoid disease. At autopsy the body was greatly wasted from the prolonged illness. Fat was found to be practically absent from the subcutaneous tissue of the parts of the body above the pelvis, but was present in moderate amount in the gluteal regions, orbits, omentum, about the kidneys, heart, pericardium and under the serous membranes. The authors felt, that had the child died from some disease not associated with extreme emaciation there would have been much more fat remaining in the regions not affected by the lipodystrophia. Microscopical sections of one ovary, the pituitary gland and both suprarenal glands showed nothing remarkable, and there was no microscopic subcutaneous fat found in a piece of the anterior abdominal wall, nor in a piece of the hairy scalp taken from the occipital region. The thyroid was relatively rather large and gave macroscopic and microscopic evidence of over-secretion of colloid material; but this did not exceed in amount that physiologically present at the age of puberty. The thymus had almost entirely disappeared. The brain was normal. There was no evidence of tuberculosis.

Etiology.—The cause of lipodystrophia is unknown. Tuberculosis, syphilis and alcoholism may be disregarded as factors. The disease is not hereditary. There is no racial predilection. The age of onset is usually about the sixth year, or shortly before puberty, and, as already shown, females are more frequently and characteristically affected than males.

Spear [13] thinks the condition closely related to the muscular dystrophies. Most authorities, however, concur with Simons [1] and Feer [12] in the opinion that it is due to a disturbance of secretion of the endocrine glands. Boissonnas [8] believes an obscure lesion of the central nervous system to be the cause. He finds it difficult to explain how a glandular lesion can, at the same time, account for the absence of fat in one part of the body and an excess of it in another; and adduces in support of his view the admission of both Simons and Feer that, whatever the cause, the nervous system must be, at least, a contributing factor. Herrman [9] thinks it quite possible that some substance circulating in the blood could produce just such opposite conditions as are met with in progressive fat dystrophy. He refers to the selective action that some organisms, toxins, and other substances have for certain tissues of the body.

Feer emphasizes the possibility of thyroid dysfunction as causative. He calls attention to the thyroid influence on the panniculus adiposus in thyrogenous obesity, on the one hand, and to the fat deficit in Basedow's disease on the other. He further alludes to the myxedematous state of the subcutaneous

others. In this condition the elastic fibers are described as elongated, but unchanged in number and diameter. The derma itself is found to be undeveloped or myxomatous; the fibrous connecting tissue is described by some investigators as absent, by others, as greatly modified; the nerves and blood-vessels are elongated and winding, and, according to Williams and Unna, there is an increase in the muscle fiber. Unna has found the marked splitting of the collagenous substances which he regards as an important factor in producing the usual extensibility of the skin. (Stelwagon, Diseases of the Skin, W. B. Saunders Co., 1916, 636; *also*, Diseases of the Skin, Ezekiel Hartzell, J. B. Lippincott Co., 1917, 518.)

connective tissue in athyrosis. The decided increase in the subcutaneous fat in certain parts of the body following castration in both sexes, and the general adiposity associated with hypoplasia of the genitalia as seen in the Froelich syndrome, are further examples of endocrinopathic influence on the panniculus adiposus.

Other authors, besides those last mentioned, have noted in their cases certain symptoms which are usually interpreted as suggestive of over-function of the thyroid gland. It is well therefore to study carefully all cases of lipodystrophia with reference to this gland as a possible factor of the disease. In my own case there are present, moderate enlargement of the thyroid, some prominence of the eyes, slight lagging of the upper lid, moderate tachycardia, tremor of the fingers, hypertension, acrocyanosis, slight nervousness and a decided acceleration in the metabolic rate. Another case of lipodystrophia now under observation by Dr. L. F. Barker and Dr. T. P. Sprunt, shows features which might be interpreted as due to hyperthyroidism, namely, marked acceleration in the basal metabolism, hypersensitiveness to adrenalin, moderate eye signs of hyperthyroidism, fine tremor of the fingers, marked under-nourishment, but no tachycardia and no thyroid struma.

It is quite apparent that any causative relationship existing between perverted action of one or more of the endocrine glands and certain metabolic disorders cannot be postulated until we know just what are the individual and interdependent functions of these glands in maintaining the normal metabolic balance, and that without such knowledge the cause of lipodystrophia must remain obscure.

Diagnosis.—Lipodystrophia is probably not so rare a condition as it was at first thought to be. The diagnosis is based on the symmetrical wasting of the face, or face and upper parts of the body associated in females, and exceptionally in males, with an increase in the size of the lower parts. The good health of the patient, the biopsy findings, the negative radiographs, the retention of the normal muscle and nerve functions, the negative electrical reactions and the normal sexual development exclude the other trophic disturbances.

Tuberculosis, cancer and other wasting diseases are readily eliminated by the excellent general condition of the patient. The marked emaciated appearance of the face in an individual enjoying good health is practically pathognomonic.

It is a point of some diagnostic importance to note that the buccal fat, or sucking pad, which is lost in lipodystrophia, is retained in infantile atrophy. The preservation of this subcutaneous cheek fat accounts for the rotundity of the face not infrequently seen in children otherwise greatly emaciated in advanced pulmonary tuberculosis.

The outlook in regard to the life of the patient is quite favorable. Death has been reported in two cases which was due to intercurrent disease. The atrophy in Campbell's [11] case progressed at the rate of about 2.5 cm. yearly. After a period, varying from 10 to 20 years, the maximum of the fat atrophy is reached. The spontaneous reappearance of fat in the lean parts has not been recorded. The increase in the lower part of the body also seems to remain at a standstill after progressing for a certain number of years.

The *treatment* has been unsuccessful. It has included the administration of thyroid, pituitary and ovarian extracts, massage, electricity, hydrotherapy and overfeeding. In the case of Pic and Gardère [22] rest and feeding resulted in some increase of the fat in the lower extremities, but the atrophied areas remained unchanged. Holländer [21] obtained temporary cosmetic improvement in Simons' [1] first case by the subcutaneous injection in the face of a mixture of human and sheep fat, and transient improvement was also obtained in Christiansen's [19] case by the injection of paraffin.

REPORT OF A CASE

The patient, Mrs. C. R., aged 30 years (F. 67788), is an intelligent woman of German descent. She was first seen by me on February 12, 1920, in the Out-Patient Department of The Johns Hopkins Hospital. She complained of sunken cheeks and thinness of her neck, body and arms. The object of her visit was to see if something could be done to "fill out" her cheeks. She had been told by her private physician that she had "muscular atrophy."

Family History.—Her father died of tuberculosis of the lungs. Her mother died of cancer of the stomach. The patient is the youngest child of a family of ten. Her birth was normal. Three brothers and three sisters died during infancy from diseases unknown to patient. Two sisters are living and well. One brother, previously tuberculous, is now in good health. None is excessively thin or fat and the patient says that there has been no affection in the family similar to her own.

Personal History.—The patient had measles and mumps in childhood. Menstruation began at the age of 15 years and has been normal. She is the mother of a healthy boy and has not miscarried. She has drunk beer in moderation. She tells me that she was treated in The Johns Hopkins Hospital Dispensary in 1904, and that because of her emaciated appearance, it was thought at that time that she probably had consumption of the lungs. In 1917 she again reported at the Dispensary complaining of a "cold." The diagnosis then was, "Chronic nephritis and pulmonary tuberculosis (largely fibroid)." She has never had a continuous cough, chest pain, or spitting of blood. She has frequently been regarded as cancerous or consumptive by her neighbors and friends. Her health, however, has been exceptionally good, and she has never failed to do her household work.

The wasting began in her face when she was a rosy, round-cheeked girl, about 14 years of age. It was accompanied by a vague aching in her face, sleepiness and a feeling of general weakness. These symptoms soon passed off, but the wasting progressed slowly downward, ending abruptly at the pelvic bones and groin folds. Her face, neck, thorax, upper extremities and abdomen became involved in the order named. Within three months' time from the onset, her face (she says) reached the present degree of emaciation. She does not know how long it took to grow thin from her face to her groins, but she recalls that she became fatter in the buttocks and lower extremities shortly after her face began to waste. She feels perfectly well and regards herself as stronger than most women, although she tires rather easily and is somewhat nervous at times. Her appetite and digestion are good and her bowels are regular. She voids once during the night after retiring. In spite of her facial disfigurement, she is cheerful and happy in her home.

Physical Examination.—Height, four feet, eleven and a half inches (151 cm.), without shoes: weight, 101 pounds (46 kg.), with clothes.

Appearance.—The striking feature at a glance is the emaciated appearance of the temples, face, neck, thorax, abdomen and upper extremities, contrasting sharply with the plumpness of the lower part of the body which begins abruptly at the groin folds and pelvic bones and includes the buttocks, thighs and calves. The

ankles and the feet are not abnormal. The facial wasting gives the patient an old, uncanny, even cadaverous look. Smiling produces deep folds under the zygomas and around the angles of the mouth. There is slight prominence of the eyes and of the thyroid gland, and marked prominence of the muscles of the face, neck, trunk and upper extremities.

Skin.—The skin is soft, not inelastic and of normal color except for moderate pallor of the face. There is no dermatographia. The hair growth is normal. Over the affected parts the skin can be easily picked up in thin folds and stretched away from the underlying muscles. Over the buttocks, thighs and calves it is firm and dimples on pinching. The skin-fold of the cheeks measures 3.5 mm., and of the left buttock 45 mm.

*Measurement of Skin-folds.**—Cheeks 3.5 mm., breasts 3 mm., neck 3 mm., mid right arm 3 mm., mid left arm 2.5 mm., mid right forearm 3 mm., mid left forearm 2 mm., upper back 4 mm., lower back 5 mm., abdomen 4 mm., right buttock 40 mm., left buttock 45 mm., outer, mid right thigh 25 mm., left thigh 31 mm., right calf 19 mm., left calf 35 mm.

Microscopical Examination of Skin.—A piece of skin with underlying tissue down to and including the fascia was received in formalin. It was cut from the left upper forearm. A portion was immediately transferred to Zenker formol and another to Marchi's fluid. Later, frozen sections were made from the formalin tissue and stained with sudan, with hæmatoxylin and eosin, with Wright's stain and with Weigert's elastica stain. Sections from Marchi's fluid were stained with safranine and also with the elastic stain and Mallory's connective-tissue stain. These were repeated with the Zenker section which also gives an especially brilliant Wright's or Giemsa's stain.

The skin itself was normal in appearance in gross except that the subcutaneous tissue was loose and soft in texture, grey and devoid of fat. The fascia, which was only a tiny fragment, seemed normal. Sections stained by the above methods show the following: The epidermis appears to be normal. The epithelial cells are in a uniform layer with normal papillæ of the corium and the normal arrangement of hair follicles. In one section there was a minute area in which the epithelium was somewhat pigmented, and in the interpapillary areas there were groups of pigmented cells. Elsewhere the corium shows no accumulations of cells except in one or two papillary projections which are infiltrated with mononuclear cells. Most of these are small lymphoid cells, with no granules, but there are a few scattered eosinophile mononuclear cells. Everywhere through the corium in much greater numbers than in the normal specimens used for comparison, there are twisting and writhing elongated cells which appear to be wandering in the crevices of the connective tissue, especially about the blood vessels and sweat glands, but also about the sebaceous glands. These have a relatively small nucleus and an elongated cell body full of basophile granules (mast cells). The corium itself is not abnormal; the elastic tissue fibrils run in alternate directions in successive layers and are very well developed. The hair follicles are normal in all respects and the sebaceous glands are quite normal. They contain much doubly refractive fat in large and small globules, which shines with a marble-like luster under the polarizing microscope. It stains black with osmic acid and red with sudan. Some of it is stained adhering to the hairs as they lie in the root sheaths. The sweat glands are abundant and normal. The tissue just below the corium is extremely loose and is composed of fine, wavy, connective tissue fibrils by no means so coarse as those of the corium, and a few delicate elastic fibrils. There are, as in the corium, blood vessels and some small nerves which appear to be normal. The deeper layers of this tissue contain more vessels of larger size, of which the arteries show a distinct elastic lamella. The veins show no obvious elastica but their endothelium appears to be rather swollen and extremely transparent. No fat is found in the upper layers of this tissue and it is only in its deepest layers that one encounters several small groups of three or four fat cells which are distended with fat globules. This stains brilliantly with osmic acid or sudan. It is not possible to distinguish which of the other cells about these and supplied by the same small blood vessels are really fat cells, since none of them have any very characteristic form and none contain oil droplets. The fascia does not seem to be abnormal.

Summary.—The skin is normal in histological structure except that there is an almost complete absence of fat in the tissue beneath the corium. Fat persists in the sebaceous glands where part of it is doubly refractive. There are very numerous mast cells in the corium and even in lower layers of the tissue. (W. G. MacCallum.)

Muscles.—The muscles are everywhere normally developed and are strong to test. In the lean areas they are plainly outlined when put in action. There is no fibrillary twitching. The reaction to electrical stimulation is normal.

Extremities.—Because of the absence of the subcutaneous fat, the upper extremities, though well muscled, appear markedly wasted when compared to the plump lower extremities. The hands seem atrophied. They are moist, clammy and somewhat cyanotic. There is slight tremor of the fingers. The buttocks, outer thighs and calves are well padded with subcutaneous fat. The knees are dimpled, and the ankles and feet are normal. In a word, the panniculus adiposus seems to have slipped from the upper part of the body to the lower extremities, there being a line of sharp demarcation between the areas of fat atrophy and fat hypertrophy at the upper margin of the pelvic bones and at the groin folds. The circumference of the left calf is greater than that of the right.

Measurements.—Head, 49.5 cm.; neck 28.5 cm.; chest (over mammary glands) 77 cm.; biceps, right, 21.5 cm.; left, 20 cm.; forearm, right, 21 cm.; left, 21 cm.; wrist, right, 13.5 cm.; left, 13.75 cm.; waist, 58.5 cm.; around crests of ilia, 68 cm.; buttocks, greatest dimension, 85.5 cm.; thigh, right, 49.5 cm.; left, 48.5 cm.; calf, right, 31.5 cm.; left, 32.5 cm.; ankle, right, 19 cm.; left, 19 cm.

Bones and Joints.—The skeleton is normal. Radiographs of the head show the sella to be normal in size. There is no abnormality of the small or large joints.

Glands.—There is no enlargement of the lymph nodes. The thyroid gland is somewhat enlarged. The radiograph shows no evidence of the thymus.

Thorax.—Symmetrical. Expansion full and equal. The intercostal spaces are well marked. The clavicles, scapulæ, spinous processes of the vertebræ and muscles are prominent. The breasts are pendulous and the skin covering them devoid of subcutaneous fat. The lungs are negative on physical and roentgenographic examination. (Frederick H. Baetjer, roentgenographer.)

Cardiovascular.—The heart is normally situated. The sounds are clear at the apex and base. The aortic second sound is sharply accentuated. The radials and brachials are distinctly sclerosed. The pulse, with the patient in the recumbent posture, is 80 to the minute, regular in rate and rhythm. The systolic blood pressure is 204 and the diastolic, 84. At a subsequent examination the pulse and blood pressure were recorder five times at intervals of 15 minutes. The pulse ranged from 92 to 104 per minute, and the systolic pressure, from 212 to 220. The patient was somewhat excited at the time and because of her hypertension it was thought best to omit the adrenalin test.

The Blood.—The Wassermann blood test is negative; hæmoglobin, 82 per cent; leucocytes, 12,200; red blood cells, 5,284,000; smear, Wilson stain, slight anisocytosis and poikilocytosis; color index about 1; no parasites; platelets increased in size and markedly increased in number. There is a moderate leucocytosis with a slight increase in

* The method for measuring the folds of the skin as suggested by Oeder was used. A fold of skin is raised from the muscles by an assistant who holds it between the thumbs and index fingers, the grasping fingers being held about 3 or 4 cm. apart. This double layer of skin and subcutaneous fat is then measured with an ordinary mechanic's caliper. (G. Oeder, Med. Klin., 1910, VI, 657.)

FIG. 1.—Author's patient at the age of 12 years, two years before the onset of facial wasting.

FIG. 2.—Lipodystrophia progressiva. Author's patient, age 30 years. Front view. Notice the furrows produced by smiling.

FIG. 3.—Side view of face.

FIG. 4.—Side view showing facial wasting and prominence of muscles of upper extremity.

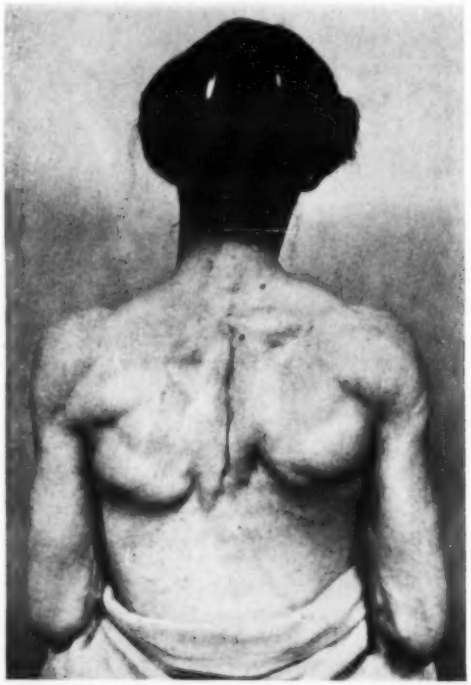

FIG. 5.—Back view.

FIG. 6.—Showing emaciation of face, trunk and upper extremities and plumpness of buttocks, thighs and legs.

FIG. 7.—Front view.

FIG. 8.—Back view.

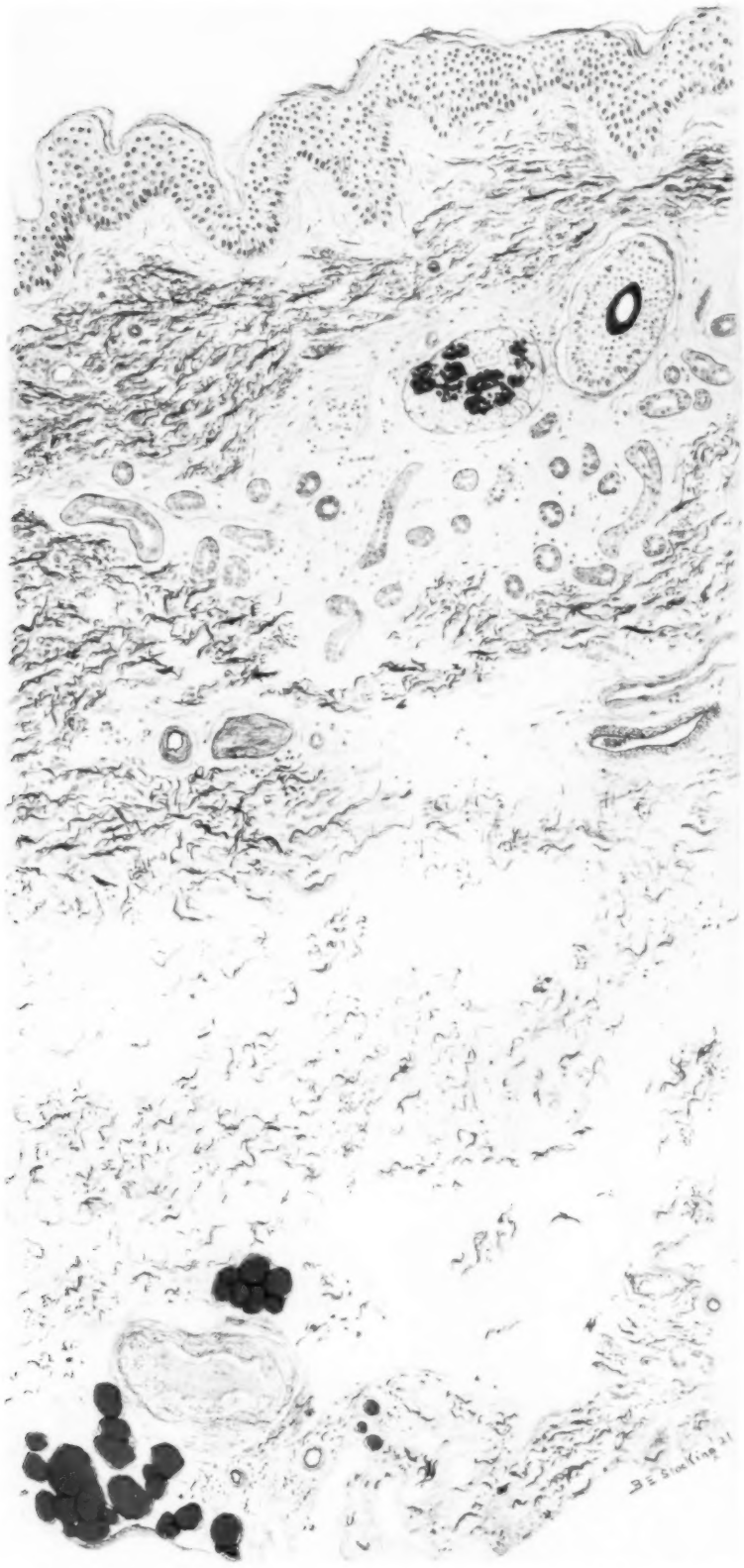

FIG. 9.—Microscopical section of a piece of skin, removed from left forearm of author's patient, showing corium and subcutaneous tissue with almost complete absence of subcutaneous fat. Fat stained with sudan.

FIG. 10.—Control illustration, after Simons showing abundance of subcutaneous fat in a section from the scalp of a healthy but not obese man, aged 34 years. Fat stained with sudan.

the polymorphonuclears. With the exception of moderate leucocytosis and the increase in the platelets, the blood picture is normal. (F. D. Conroy.)

Blood Chemistry.—The blood chemistry reveals a normal condition. The blood CO_2, 59.4 volumes per cent; the blood urea, 18.1 milligrams urea nitrogen per 100 c. c. (Walter W. Palmer.)

Blood Sugar.—The blood sugar curve is normal. (Leslie N. Gay.)

Abdomen.—The abdomen is scaphoid; walls, soft; no tenderness; no masses. The liver and spleen are not palpable. The double skinfold of the abdomen, as stated, measures 4 mm.

Digestive System.—The color of the lips and mucous membrane is normal. The gums are in good condition. The teeth are sound; several molars are missing; one molar is capped. The tongue is clean and is protruded in the mid-line without tremor. Fluoroscopic examination reveals visceroptosis with normal stomach. Examination of the gastric contents after a rice test meal shows normal findings. The stool is normal in appearance. There are no ova; no occult blood.

Genito-Urinary.—There is no gynecological disease.

Urine.—Single specimen, clear, light straw; reaction, acid; sp. gr., 1014; heavy trace of albumin; no sugar; no casts. Twenty-four hour specimen, 995 c. c.; clear straw; acid, sp. gr., 1012; albumin present. Tests for sugar, diacetic and diazo-reaction and urobilin are negative. Microscopically, a few hyaline and granular casts; no red blood cells; an occasional white cell. Albumin and casts were present on several subsequent examinations.

Renal Function.—There is a phenolsulphonephthalein output of 21 per cent, first hour; second hour 12 per cent.

Basal Metabolism.—The metabolic rate shows a decided acceleration. The CO_2 output per square meter of body surface per hour is 36.8 per cent above the expected average for the patient's age and sex. (John T. King, Jr.)

Eye, Ear, Nose, Sinuses and Throat.—There is slight protrusion of the eyes. Right pupil is larger than the left. Both react promptly to light and accommodation. The eye movements are normal in all directions. No nystagmus. The convergence is normal, and except the slight exophthalmos and slight lid lag, there are no signs suggesting hyperthyroidism. The fundi show a low grade of optic neuritis and definite sclerosis of the retinal vessels. The ears, nose, accessory sinuses and throat are normal.

Neurological Examination.—The musculature of the entire body is normal in development, strength and function. There is no incoordination, no sign of muscular atrophy, no fibrillary twitching and no change in the electrical reactions. The fingers show a slight tremor.

The skin reveals no evidence of trophic change, but there is a marked abscence of subcutaneous fat in the face, neck, shoulders, arms, forearms, hands, chest, back, and abdomen and also, an increase of subcutaneous fat in the buttocks, thighs and legs.

The sensory and vasomotor functions are not abnormal.

The deep reflexes are active. The superficial reflexes are present with the exception of the abdominal skin reflex.

Romberg's, Trousseau's, Kernig's, and Babinski's signs are absent.

With the exception of moderate optic neuritis, the nerves of special sense are normal.

Mentality.—The patient is alert and very intelligent. (G. Lane Taneyhill.)

The case is one of lipodystrophia progressiva, associated with arteriosclerosis, chronic nephritis, hypertension, and a moderate thyropathy as shown by the slight enlargement of the thyroid gland, the rather prominent eyes, the slight lid lag, the tremor, the slight nervousness, the increase in the pulse rate, the acrocyanosis and the accelerated metabolic rate.

SUMMARY

Lipodystrophia progressiva is a relatively rare condition, beginning insidiously and usually in early life; caused, possibly, by endocrine dysfunction; not hereditary; does not endanger life; and, is more commonly and more characteristically developed in the female. It is characterized pathologically by a slowly progressive, almost complete and probably permanent disappearance of the subcutaneous fat from the head, face, neck, upper extremities, and from the trunk as far as the pelvic bones and groin folds, and, especially in the female, by an increase in the subcutaneous fat of the buttocks, thighs and legs.

In conclusion I wish to express my thanks to Professor William G. MacCallum for the histological examination of the specimen of skin from my patient, and also for the excellent drawing of a microscopic section of the skin which accompanied the report. I am further indebted to Dr. G. Lane Taneyhill, Jr., for the neurological examination and for having pointed out the nature of the case, and to others for special examinations.

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AN EXPERIMENTAL STUDY OF PHAGOCYTOSIS IN RELATION TO TERMINAL INFECTIONS

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Introduction

The circumstances which originally suggested this investigation were the instances of energetic phagocytosis frequently observed at autopsy. The surprising number of bacteria occasionally found within the leucocytes in individuals dying of fatal infection or intoxication suggests the possibility that the diminution of phagocytic activity sometimes associated with destructive infections may not be as nearly universal as is commonly supposed. It further seems probable that in moribund animals, "in which the resistance fails,"[1] the phagocytic defense may remain unimpaired, and, occasionally at least, function at a level considerably above the normal. These observations also indicate that terminal infections cannot be accounted for by assuming a collapse or a decrease in the activity of the phagocytic functions of the animal.

Fig. 1 illustrates the extent of phagocytosis which may occur in animals dying of fatal infections.

Historical Sketch

The widespread occurrence of terminal infections was strikingly expressed by Osler when he wrote that, "Persons rarely die of the diseases with which they suffer."[2] However, the nature of the rupture in the defensive mechanism of the individual which permits the rapid invasion of the body before death by even the feebly aggressive bacteria, has never been satisfactorily explained. Flexner[3] undertook a study of this condition as early as 1896. He, however, limited his investigations to an examination of the humoral defense without any attempt at ascertaining whether cellular modifications might not be a contributing cause in terminal infections. He thought that he discovered, at least in a few cases, a slight decrease in the bactericidal actions of human serum against staphylococci. The correctness of these findings, however, was soon placed in doubt by Wright,[4] who, together with Windsor, demonstrated in 1902 that human blood exhibits an almost total absence of any bactericidal influence against staphylococci.

Bordet[5] was one of the first to suggest that the phagocytic activity of the body might be a relatively stable function and not easily influenced by conditions which profoundly affect other vital activities. He demonstrated that deep chloroform anesthesia which "completely deadens"[5] the central nervous system has no disturbing influence upon phagocytosis. This investigator also observed that diphtheria toxin exerts little, if any, effect on phagocytic activity.[6]

Tunnicliff,[7] in an investigation of the opsonic index during the leucopenia resulting from measles, found that there was a slight decrease for streptococci, staphylococci, and tubercle bacilli. She suggested that the decrease in phagocytic activity against these organisms might "account for the secondary infections" commonly associated with measles.

Bartlett and Ozaki,[8] in 1917, injected a dog with a massive dose of *B. coli* and five hours later, when the animal was in a moribund condition, inoculated it with a quantity of staphylococci. They observed no decrease in the opsonic index for staphylococcus. This experiment was not repeated and the authors themselves were not entirely convinced of the adequacy of their controls.

There is, of course, an extensive literature (Metchnikoff;[9] Wright;[10] Bull;[11] Hektoen;[12] Opie,[13] and others) indicating a sharp and very considerable decline, in some instances at least, in the phagocytic activity of the body against the specific infecting organism in fatal infections. There seems to have been no organized inquiry, however, concerning the opsonic index for those bacteria which take no part in the original infection but which later invade the weakened body giving rise to the destructive phenomenon known as terminal infection.

Bacterial invasion of the wasted body immediately preceding death is sometimes so complete and sudden that it has been assumed the process proceeds unopposed. The collapse of the defensive mechanism against bacteria in terminal infections seems to be thorough and complete. Zinsser[14] expressed this idea in a discussion of fatal infections when he wrote: "The infectious process becomes rapidly generalized, the bacteria enter the blood-stream and lymphatics, and the defensive powers are overwhelmed." The possibility of a distinct part

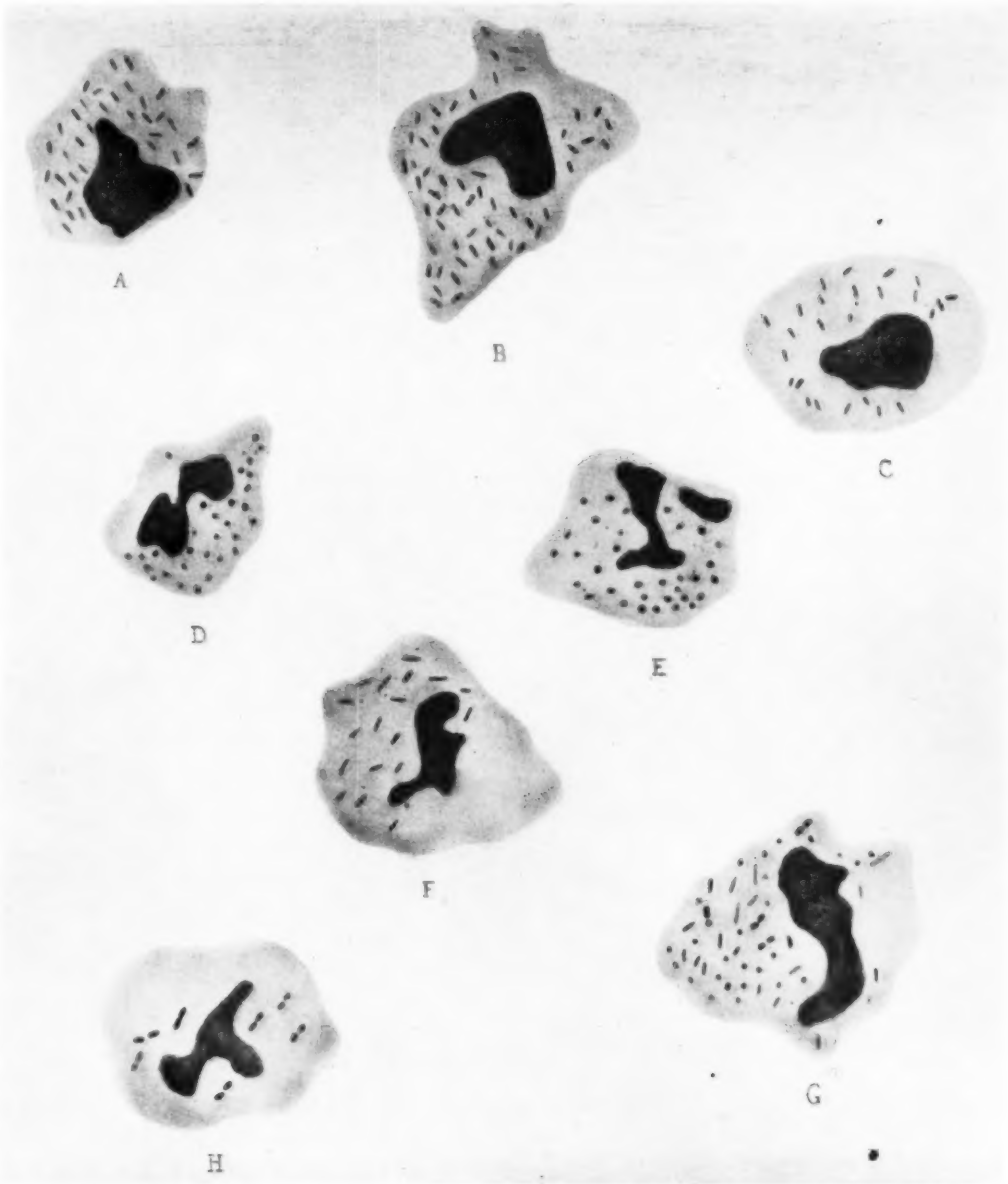

FIG. 1.—Illustrating the extent of phagocytosis which may occur during the late states of terminal infection. *A* and *B*. Cells from the peritoneal cavity of a guinea-pig dying of *B. coli* peritonitis. *C*. Phagocyte from lung of patient dying of influenza pneumonia. *D*. Leucocyte from peritoneal cavity of a guinea-pig dying of staphylococcus peritonitis. *E*. Leucocyte from blood stream of a guinea-pig dying of generalized staphylococcus infection. *F*. Phagocyte containing diphtheria bacilli from lung of child with terminal pneumonia following diphtheria. *G*. Cell containing mixed flora from lung of patient dying of terminal pneumonia. *H*. Phagocyte from pleural exudate of a boy dying of generalized pneumococcus infection.

of this protective adaptation of the body remaining intact, with unimpaired function, has not been believed and awaits confirmation. It is proposed, therefore, in this research to undertake an investigation of the phagocytic activity in animals after kataphylaxis * has occurred, and to determine what change, if any, occurs in the opsonic index for those organisms which most frequently overrun the body late in fatal infections and intoxications.

PLAN OF INVESTIGATION

The investigation of this problem has been done almost entirely *in vitro* because there is no thoroughly dependable method of controlling results when the experiments are undertaken *in vivo*. Wright's [1] work has established that even slight changes in the phagocytic activity of the animal can be demonstrated by *in vitro* methods, so that if there is a decline in opsonic power sufficient to account, even in part, for the bacterial invasion occurring in terminal infections, there could be no difficulty in demonstrating that decrease in effectiveness by the methods employed in this investigation. The bacteria used were those most commonly associated with terminal infections—a pyogenic coccus, *Staphylococcus aureus,* recently isolated from a case of human furunculosis, and a Gram-negative bacillus, *B. coli communis*. In addition, two other organisms were commonly used. *B. bronchisepticus* was selected because it is readily taken up in large numbers, making possible dependable counts in weakly phagocytic cells, while *B. typhosus,* resisting engulfment to considerable extent, permits reliable enumeration when phagocytosis with other bacteria is so vigorous that even a depressed phagocytic capacity is quite sufficient to fill the cells beyond counting. Whenever possible the specific infecting organism was included in each series of experiments.

TECHNIQUE

The opsonic technique used in this research was essentially the same as that originally employed by Wright and Douglas.[2] Certain modifications and refinements, however, were introduced which seemed to insure greater uniformity in the results, and to render this generally confusing technique more dependable and satisfactory.

The blood providing the cells was received into a centrifuge tube containing several volumes of citrated salt solution. Before sedimentation the cells were uniformly suspended by repeated suction and ejection with a pipette. This process was repeated before each centrifugation and is a most effective means of preventing the aggregation of the platelets around the leucocytes. The centrifugation was accomplished at a speed which permitted the sedimentation of the white cells in four to five minutes without throwing down the platelets. This is most important, for it eliminates the platelets from the leucocytic layer and prevents packing of the white cells. The washings were repeated three times and the leucocytes suspended in a volume of physiological salt solution equal to one third the original volume of blood. After standing for one hour, cells were uniformly suspended by a gentle continued agitation of the tube and all samples of any one experimental series were taken immediately. It was early observed that the ingesting capacity of phagocytes was not always so reliable and vigorous if they were used after washing as when they were allowed to stand for an hour. This is probably occasioned by a disturbance in the osmotic pressure resulting from repeated washings and centrifugation.

Confusing and contradictory results invariably accompanied a careless or hurried preparation of the bacterial suspension. The organisms used in this work were grown, whenever possible, on moist plain agar for 15 hours. The tube was then washed out with saline to remove lint and debris. Five cubic centimeters of salt solution were next added and the tube gently agitated until the resulting turbidity was equal to or slightly in excess of that desired. This was then transferred to a second clean tube and vigorously shaken to break up possible clumps. Finally, the tube was centrifuged at a speed sufficient to remove from suspension all but the single bacteria. Two and five-tenths cubic centimeters were then drawn from the upper portion and reserved for the bacterial suspension. All the samples for any experimental series were regularly taken from the same level in order to insure a more nearly uniform number of organisms. Bacteria which do not grow upon plain agar or which could not be uniformly suspended from solid media were grown in meat infusion broth. For growing massive cultures of pneumococci, whole blood was added to the broth. The bacteria were sedimented, washed and suspended in salt solution. A turbidity was selected which presented a delicate opalescence in indirect light.

Sera were taken in the usual manner except that those containing an excessive amount of fat were, whenever possible, avoided. Those containing agglutinins for the experimental cells, although thoroughly annoying, can be used without invalidating the results. Although a difference of a few hours in the age of the sera results in no demonstrable difference in their opsonic reactions, all sera for an experimental series were collected as nearly as possible at the same time.

The phagocytic mixtures were prepared according to the Wright technique, and incubated at 37° C. for 20 minutes. The smears and stains were made in the manner described by Cross.[3] All smears showing gross differences in the number or distribution of the leucocytes were discarded and the preparations repeated. The organisms in 50 polymorphonuclear leucocytes were counted and these cells were always enumerated from corresponding areas on the control and experimental slides. Cells containing an excessively large number of bacteria show a tendency to collect in portions of the smear which can be predicted, and the most confusing and contradictory deviations present themselves if the selection of corresponding

* The word kataphylaxis was introduced by Bullock and Cramer (Proc. of the Royal Society, 1919, Series B, vol. 90, p. 513) and was defined by them as a rupture in the local defensive mechanism against bacteria. As used in the present paper, the word will be understood to indicate a break-down in the general defensive mechanism sufficiently complete to permit the infection to lead uninterruptedly to the death of the animal.

areas for enumeration is not rigorously observed. All preparations exhibiting marked variations from the normal or expected were repeated throughout. Whenever the phagocytes revealed unusual inequalities in the number of ingested organisms, parallel preparations were made and the average enumeration taken as the true count. Polymorphonuclear leucocytes alone were considered, and no attempt was made at enumeration in cells containing more than 25 organisms. In all counts the percentage of ingesting leucocytes as well as the total number of intracellular bacteria were determined.

Subject Matter

The data presented in this paper are selected from a study of 85 cases of infection which resulted in death. An effort was made to secure the widest possible variety, including both spontaneous and induced infections. The period elapsing between the primary inoculation and death ranged from a few hours to several weeks. The arrangement of subject matter and condition of each experiment are clearly set forth in the various protocols.

Experimental Data

PART I

A. *Pneumococcus Infections*

Experiment 1.—The dog, No. 11, used in this experiment was an adult female weighing 15½ pounds. It was inoculated intravenously on November 27, 1920, with a sublethal dose of virulent pneumococcus, Type I. The sublethal doses were continued on dates indicated in the protocol until the blood exhibited a high bacteriotropic action. The intravenous inoculations were then gradually increased until a sufficient dose was administered to bring about the death of the animal. This result was hastened toward the end of the experiment by the injection of pneumococci into the left pleural cavity. A sample of blood was taken before each injection and allowed to clot. Two hours later the serum was withdrawn and the opsonic index determined in the manner described above. The normal dogs providing the leucocytes and control serum were always bled at the same time as the experimental animal. The results of all opsonic determinations are included in the following tables:

Date	Remarks	Bacteria.	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
11-27-20	Dog No. 11. Before injection wt. 15½ lbs. Inj. 25 c. c. Pn. I.	S. aureus....	No. 11	50	39	11	145	2.9*
			Con.	50	42	8	121	2.4
		Pn. I.....	No. 11	50	1	49	2	0.0
			Con.	50	4	46	7	0.1
		B. coli	No. 11	50	41	9	86	1.7
			Con.	50	40	10	93	1.8
		B. typhosus.	No. 11	50	43	7	95	1.9
			Con.	50	45	5	104	2.0
11-29-20	Dog No. 11. Wt. 14½ lbs. Inj. 50 c. c. Pn. I.	S. aureus....	No. 11	50	42	8	175	2.5
			Con.	50	43	7	171	3.4
		Pn. I.....	No. 11	50	0	50	0	0.0
			Con.	50	0	50	0	0.0
		B. coli	No. 11	50	43	7	96	1.7
			Con.	50	46	4	104	2.0
		B. typhosus.	No. 11	50	35	15	66	1.3
			Con.	50	38	12	70	1.4
12-1-20	Dog No. 11. Wt. 13½ lbs. Inj. 100 c. c. Pn. I.	S. aureus	No. 11	50	41	9	220	4.4
			Con.	50	44	6	212	4.2
		Pn. I.....	No. 11	50	26	24	101	2.0
			Con.	50	5	45	6	0.1
		B. coli	No. 11	50	19	31	50	1.0
			Con.	50	25	25	55	1.1
		B. typhosus.	No. 11	50	13	37	21	0.4
			Con.	50	15	35	20	0.4
12-3-20	Dog No. 11. Wt. 14 lbs. †Inj. 150 c. c. Pn. I.	S. aureus....	No. 11	50	24	26	57	1.1
			Con.	50	27	23	60	1.2
		Pn. I.....	No. 11	50	31	19	100	2.0
			Con.	50	2	48	4	0.1
		B. coli	No. 11	50	23	27	28	0.5
			Con.	50	24	26	35	0.7
		B. typhosus.	No. 11	50	39	11	82	1.6
			Con.	50	42	8	85	1.6
12-10-20	Dog No. 11. Wt. 13 lbs. Inj. 200 c. c. Pn. I.	S. aureus....	No. 11	50	41	9	106	3.3
			Con.	50	38	12	145	2.9
		Pn. I.....	No. 11	50	38	12	162	3.2
			Con.	50	8	42	22	0.4
		B. typhosus.	No. 11	50	21	29	30	0.6
			Con.	50	20	30	45	0.9
12-17-20	Dog No. 11. Wt. 13 lbs. Inj. 250 c. c. Pn. I.	S. aureus....	No. 11	50	40	10	269	3.3
			Con.	50	43	7	231	4.6
		Pn. I.....	No. 11	50	25	25	75	1.5
			Con.	50	10	40	21	0.4
		B. coli	No. 11	50	32	18	77	1.5
			Con.	50	33	17	80	1.6
		B. typhosus.	No. 11	50	32	16	70	1.4
			Con.	50	38	12	86	1.7
12-22-20	Dog No. 11. Wt. 13 lbs. Inj. 250 c. c. Pn. I.	S. aureus....	No. 11	50	33	17	121	2.4
			Con.	50	25	25	104	2.0
		Pn. I.....	No. 11	50	25	25	161	3.2
			Con.	50	0	50	0	0.0
		B. coli	No. 11	50	43	7	113	2.2
			Con.	50	38	12	105	2.1
		B. typhosus.	No. 11	50	30	20	50	1.0
			Con.	50	31	19	68	1.3
12-26-20	Dog No. 11. Wt. 13 lbs. Inj. 250 c. c. Pn. I.	.....	.....	....	....	....	....	....
12-30-20	Dog No. 11. Wt. 13 lbs. Inj. 250 c. c. Pn. I.	.....	.....	....	....	....	....	....
1-10-21	Dog No. 11. Wt. 13 lbs. Inj. 250 c. c. Pn. I.	.....	.....	....	....	....	....	....
1-10-21	Dog No. 11. Wt. 13 lbs. Inj. 250 c. c. Pn. I.	S. aureus....	No. 11	50	37	13	199	3.9
			Con.	50	33	17	180	3.6
		Pn. I.....	No. 11	50	22	28	62	1.3
			Con.	50	8	42	16	0.3
		B. coli	No. 11	50	41	9	163	2.0
			Con.	50	45	5	140	2.8
		B. typhosus.	No. 11	50	26	24	27	0.5
			Con.	50	23	27	38	0.7
1-20-21	Dog No. 11. Wt. 13 lbs. ‡Inj. 245 c. c. Pn. I.	S. aureus....	No. 11	50	36	14	146	2.9
			Con.	50	35	15	171	3.4
		Pn. I.....	No. 11	50	0	50	0	0.0
			Con.	50	0	50	0	0.0
		B. coli	No. 11	50	42	8	93	1.9
			Con.	50	41	9	95	1.9
		B. bronchisept.	No. 11	50	27	23	65	1.3
			Con.	50	42	8	73	1.4
1-22-21	‡Inj. 50 c. c. Pn. I.	.....	.....	....	....	....	....	....
1-24-21	Dog No. 11. Wt. 11 lbs. No inj.	S. aureus...	No. 11	50	34	16	128	2.5
			Con.	50	31	19	115	2.3
		Pn. I.....	No. 11	50	0	50	0	0.0
			Con.	50	0	50	0	0.0
		B. coli	No. 11	50	41	9	78	1.5
			Con.	50	41	9	71	1.4
		B. bronchisept.	No. 11	50	37	13	136	2.7
			Con.	50	38	12	137	2.7
1-25-21	Dog No. 11. Wt. 11 lbs. This reading made 10 hours before death. No injection.	S. aureus....	No. 11	50	35	15	235	4.7
			Con.	50	32	18	199	3.9
		Pn. I.....	No. 11	50	4	46	8	0.1
			Con.	50	0	50	0	0.0
		B. coli	No. 11	50	15	35	45	0.9
			Con.	50	14	36	50	1.0
		B. bronchisept.	No. 11	50	39	11	212	4.2
			Con.	50	37	13	196	3.9
		B. typhosus.	No. 11	50	7	43	15	0.3
			Con.	50	8	42	19	0.3
1-25-21	Dog No. 11. Wt. 11 lbs. This reading made at the time of death.	S. aureus....	No. 11	50	34	16	351	5.0
			Con.	50	33	17	199	3.9
		Pn. I.....	No. 11	50	7	43	15	0.3
			Con.	50	0	50	0	0.0
		B. coli	No. 11	50	14	37	47	0.9
			Con.	50	14	36	50	1.0
		B. bronchisept.	No. 11	50	33	17	226	4.5
			Con.	50	37	13	195	3.9
		B. typhosus.	No. 11	50	6	44	16	0.3
			Con.	50	8	42	19	0.3

* The opsonic indices which are plotted in the accompanying graphs are obtained for each microorganism by dividing the phagocytic index of the experimental animal for this bacterium by the corresponding phagocytic index of a normal control animal.

† Pneumococci were grown in meat infusion broth containing 1% whole blood. The bacteria for each injection were sedimented from the quantity indicated in the table, and then suspended in 25 c. c. of broth before inoculation.

‡ The bacteria from 25 c. c. of this inoculation were injected into the left pleural cavity. The remaining quantity was given intracardially.

Anatomical Diagnosis.—Slight emaciation, broncho-pneumonia, purulent exudate in both pleural cavities, generalized edema. Pneumococcus, Type I, cultivated in pure culture from pleural exudate and from heart's blood.

Kataphylaxis in this animal probably occurred on January 20, five days before its death, and was first indicated by the appearance of pneumococci in the blood in sufficient quantity to be recovered in the culture. By referring to the curves in Fig. 2 it will be observed that there was no post-kataphylactic

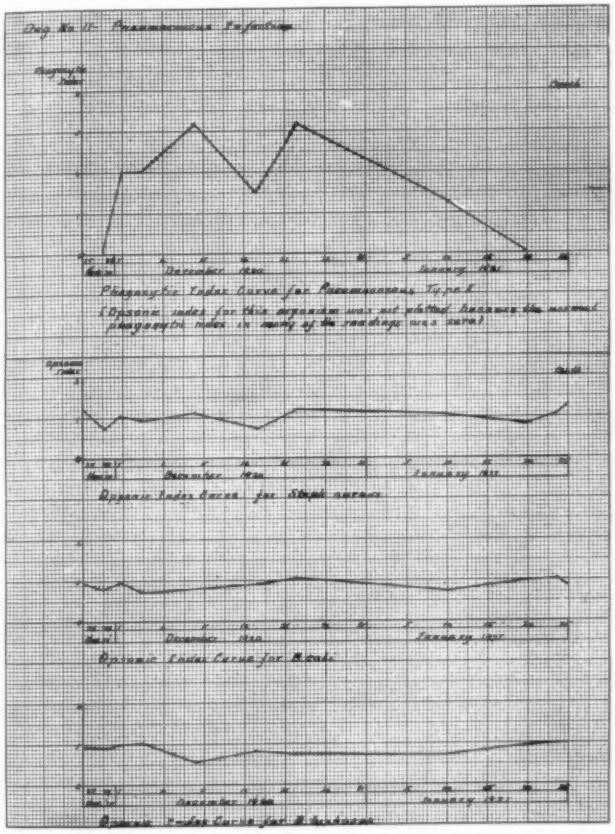
Dog No. 11. Pneumococcus Infection
Phagocytic Index Curve for Pneumococcus Type I.
(Opsonic index for this organism was not plotted because the normal phagocytic index in many of the readings was zero)
Opsonic Index Curve for Staph. aureus.
Opsonic Index Curve for B. coli.
Opsonic Index Curve for B. typhosus.

FIG. 2.

decline in the opsonic index for any except the specific infecting organism. Samples of blood taken a few moments before death, when the animal was in a state of complete collapse, revealed a phagocytic activity in all respects equal to the normal and for staphylococci it was even somewhat increased.

The opsonic index for the infecting organism behaved throughout in accordance with the findings published by Neufeld and Töpfer[18] and Rosenow.[19] Virulent pneumococci are not phagocyted at all by the normal animal, but after one or two inoculations of this organism the opsonins for pneumococci increase and can be still further increased by subsequent injections, provided a lethal dose is not administered. Once this quantity is given, however, there is a rapid decline in phagocytic activity, the zero mark ordinarily being reached some time before the death of the animal.

The variation in the opsonic curve for *Staphylococcus aureus* requires some explanation. Up to December 3 the curve for this organism had remained normal, but at this time a culture of Pneumococcus I, contaminated with *Staphylococcus aureus* was injected and five days later the dog's blood showed an increase in opsonins for staphylococci. This organism was almost immediately overcome, for it was never obtained in subsequent blood cultures. The increased opsonic activity for staphylococci continued a few days, but gradually decreased until at the end of a month the index was again normal. However, on January 20 pneumococci appeared in the blood and the reaction against this homologous antigen resulted in a non-specific increase in the residue of antibodies remaining from the previous staphylococcus inoculation. This was immediatelyy reflected by a rise in the opsonic curve for Staphylococcus. These observations are in agreement with the findings of Bieling.[20]

B. *Staphylococcus Infections*

EXPERIMENT 2.—An adult female dog, No. 14, weighing 15 pounds was selected as the experimental animal in this test. She was given an initial intravenous injection of 1 c.c. of a broth culture of *Staph. aureus* on November 27, 1920. Gradually increasing amounts of this organism were given until the dog, in an emaciated and helpless condition, died 23 days later.

The opsonic determinations, together with other data concerning this experiment are recorded in the appended table.

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic Index
11-7-20	Dog No. 14. Wt. before injunction 15 lbs. Inj. 1 c. c. S. aureus.	S. aureus....	No. 14	50	41	9	121	2.4
			Cor.	50	42	8	120	2.4
		Pn. I.....	No. 14	50	5	45	10	0.2
			Con.	50	4	46	8	0.1
		B. typhosus.	No. 14	50	42	8	106	2.1
			Con.	50	45	5	104	2.0
11-20-20	Dog No. 14. Wt. 13½ lbs. Inj. 2 c. c. S. aureus.	S. aureus....	No. 14	50	44	6	297	5.9
			Can.	50	44	6	170	3.4
		Pn. I.....	No. 14	50	0	50	0	0.0
			Con.	50	0	50	0	0.0
		B. coli	No. 14	50	38	12	96	1.9
			Con.	50	46	4	104	2.0
		B. typhosus.	No. 14	50	35	15	100	2.0
			Con.	50	38	12	70	1.4
12-1-20	Dog No. 14. Wt. 13½ lbs. No inj.	S. aureus....	No. 14	50	50	0	309	6.2
			Con.	50	44	6	212	4.2
		Pn. I.....	No. 14	50	4	46	10	0.2
			Con.	50	5	45	6	0.1
		B. coli	No. 14	50	34	16	56	1.1
			Con.	50	35	15	55	1.1
		B. typhosus.	No. 14	50	14	36	18	0.3
			Con.	50	15	35	20	0.4
12-3-20	Dog No. 14. Wt. 13 lbs. Inj. 5 c. c. S. aureus.	S. aureus....	No. 14	50	28	22	66	1.3
			Con.	50	27	23	61	1.2
		Pn. I.....	No. 14	50	3	47	7	0.1
			Con.	50	2	48	4	0.0
		B. coli	No. 14	50	31	19	50	1.0
			Con.	50	34	16	35	0.7
		B. typhosus.	No. 14	50	36	14	76	1.5
			Con.	50	42	8	85	1.6
12-7-20	Dog No. 14. Wt. 12½ lbs. No inj.	S. aureus....	No. 14	50	35	15	73	1.5
			Con.	50	34	16	150	3.0
		Pn. I.....	No. 14	50	....	....	....	....
			Con.	....	....	....	....	....
		B. coli	No. 14	50	49	1	156	3.1
			Con.	50	39	11	131	2.6
		B. typhosus.	No. 14	....	....	....	....	....
			Con.	....	....	....	....	....
12-10-20	Dog No. 14. Wt. 11 lbs. No inj.	S. aureus....	No. 14	50	41	9	76	1.5
			Con	50	39	11	115	2.3
		Pn. I.....	No. 14	50	....	....	....	....
			Con.	50	....	....	....	....
		B. coli	No. 14	50	31	19	73	1.5
			Con.	50	33	17	75	1.5
		B. typhosus.	No. 14	50	19	31	42	0.8
			Con	50	23	27	39	0.8

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
12-15-20	Dog No. 14. Wt. 11 lbs. No. inj.	S. aureus....	No. 14	50	29	21	165	3.1
			Con	50	25	25	171	3.4
		Pn. I.....	No. 14	50	....	....	....	....
			Con.	50	....	....	....	....
		B. coli	No. 14	50	38	12	87	1.7
			Con.	50	42	8	73	1.5
		B. typhosus.	No. 14	50	27	24	55	1.1
			Con.	50	28	22	46	0.9
12-17-20	Dog No. 14. No inj.	S. aureus....	No. 14	50	44	6	354	7.9
			Con.	50	43	7	231	4.6
		Pn. I.....	No. 14	50	9	41	22	0.4
			Con.	50	10	40	21	0.4
		B. coli	No. 14	50	35	15	71	1.4
			Con.	50	33	17	80	1.6
		B. typhosus.	No 14	50	40	10	92	1.8
			Con.	50	38	12	86	1.7
12-19-20 11:00 P. M.	Dog No. 14. Reading made at time of death.	S. aureus....	No. 14	50	36	14	121	2.4
			Con	50	38	12	219	4.4
		Pn. I	No 14	50	4	46	18	0.3
			Con.	50	5	45	12	0.2
		B. coli	No 14	50	41	9	111	2.2
			Con.	50	42	8	91	1.8
		B. typhosus.	No. 14	50	29	21	72	1.4
			Con.	50	30	20	43	1.0

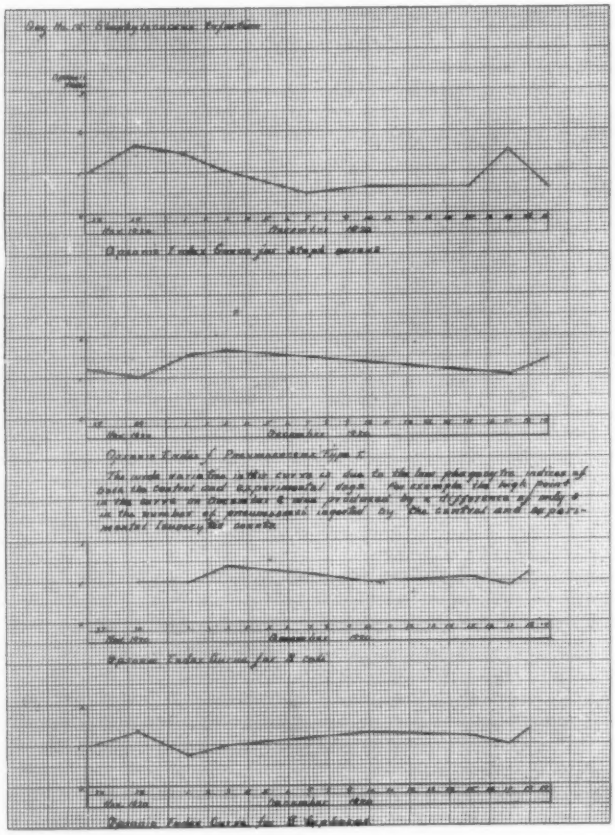

FIG. 3.

Anatomical Diagnosis.—Marked emaciation, Staphylococcus broncho-pneumonia, multiple abscesses in body wall, heart, kidneys and spleen, acute nephritis, staphylococcus cystitis. *Staph. aureus* in pure culture obtained from heart's blood, kidneys, spleen and urine.

The variations in the opsonic index for *Staph. aureus* in this experiment are in agreement with the results published by Wright[21] and Neufeld.[22] Under the stimulation of sublethal inoculation there is an increase in phagocytic activity but as soon as the lethal dose is given there is a sharp decline which often forces the index below the normal. One especially interesting feature of the opsonic index for Staphylococcus in this experiment is that on December 7th the index was somewhat lower than it was at the time the animal died. Although phagocytosis is regarded as the chief defensive mechanism opposed against staphylococci, this animal with an uncomplicated infection survived the period of its lowest phagocytic activity and died when its opsonic index was only slightly lower than the normal.

This case represents an infection of the most violent and destructive nature. The animal lost 26 per cent of its body weight, and during the last 36 hours of life was in a state of complete exhaustion and collapse. Suppurative lesions occurred generally throughout the body. In spite, however, of the overwhelming character of this infection there was no decrease in phagocytic activity, even in the hours immediately preceding death, against any organisms not concerned in the primary infection.

C. *Typhoid Infections*

EXPERIMENT 3.—In this experiment an adult male dog, No. 13, weighing 13½ pounds, was injected intravenously on November 27, 1920, with 2 c.c. of an 18-hour broth culture of *B. typhosus*. Sublethal inoculations were continued until the blood revealed a high opsonic content and then the injections were gradually increased until the dog died on December 28, 1920.

Samples of blood were taken before each injection and the usual opsonic determinations made under the conditions described in the following table:

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
11-27-20	Dog No. 13. Wt before injection 13½ lbs. Inj. 2 c. c. B. typhosus.	S. aureus....	No. 13	50	48	2	133	2.6
			Con.	50	42	8	121	2.4
		Pn. I.....	No 13	50	3	47	4	0.0
			Con.	50	4	46	8	0.1
		B. coli	No. 13	50	..	..	..	..
			Con.	50	..	..	..	..
		B. typhosus.	No. 13	50	42	8	97	1.9
			Con.	50	45	5	104	2.0
11-29-20	Dog No. 13. Wt. 13½ lbs. No inj.	S. aureus....	No. 13	50	47	3	150	3.0
			Con.	50	44	6	170	3.4
		Pn. I.....	No. 13	50	0	50	0	0.0
			Con.	50	0	50	0	0.0
		B. coli	No. 13	50	41	9	83	1.6
			Con.	50	46	4	102	2.0
		B. typhosus.	No. 13	50	43	7	95	1.9
			Con.	50	39	11	71	1.4
12- 1-20	Dog No. 13. Wt. 13½ lbs. Inj. 1 c. c. B. typhosus.	S. aureus....	No. 13	50	43	7	218	4.3
			Con.	50	45	5	212	4.2
		Pn. I.....	No. 13	50	4	46	8	0.1
			Con.	50	4	46	6	0.1
		B. coli	No. 13	50	32	18	53	1.0
			Con.	50	25	25	55	1.1
		B. typhosus.	No. 13	50	17	33	45	0.9
			Con.	50	15	35	20	0.4
12- 3-20	Dog No. 13. Wt. 13 lbs. Inj. 4 c. c. B. typhosus.	S. aureus....	No. 13	50	23	27	70	1.4
			Con.	50	27	23	63	1.2
		Pn. I.....	No. 13	50	4	46	8	0.1
			Con.	50	2	48	4	0.0
		B. coli	No. 13	50	24	26	37	0.7
			Con.	50	24	26	35	0.7
		B. typhosus.	No. 13	50	49	1	300	6.4
			Con.	50	42	8	85	1.7
12-17-20	Dog No. 13. Wt. 11 lbs. Inj. 5 c. c. B. typhosus.	S. aureus...	No. 13	50	46	4	232	4.6
			Con.	50	43	7	231	4.6
		Pn. I.....	No. 13	50	7	43	25	0.5
			Con.	50	10	40	21	0.4
		B. coli	No. 13	50	40	10	84	1.6
			Con.	50	33	17	80	1.6
		B. typhosus.	No. 13	50	42	8	211	4.2
			Con.	50	38	12	86	1.7

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
12-22-20	Dog No. 13. Wt. 11¼ lbs. Inj. 5 c. c. B. typhosus.	S. aureus....	No. 13	50	26	24	119	2.3
			Con.	50	25	25	104	2.1
		Pn. I.....	No. 13	50	0	50	0	0.0
			Con.	50	0	50	0	0.0
		B. coli	No. 13	50	41	9	130	2.6
			Con.	50	38	12	105	2.1
		B. typhosus.	No. 13	50	40	10	211	4.2
			Con.	50	31	19	68	1.3
12-26-20 9:00 A. M.	Dog No. 13. Wt. 9½ lbs. No inj.	S. aureus....	No. 13	50	45	5	236	5.1
			Con.	50	41	9	250	5.0
		Pn. I.....	No. 13	50	0	50	0	0.0
			Con.	50	0	50	0	0.0
		B coli	No. 13	50	25	25	43	0.8
			Con	50	37	37	47	0.9
		B. typhosus.	No. 13	50	27	27	77	1.5
			Con.	50	4	4	10	0.2
12-26-20 5:00 P. M.	Dog No. 13. Wt. 9 lbs. No inj.	S. aureus....	No. 13	50	35	15	227	4.5
			Con.	50	41	9	250	5.0
		Pn. I.....	No. 13	50	0	50	0	0.0
			Con.	50	0	50	0	0.0
		B. coli	No. 13	50	21	29	42	0.8
			Con.	50	37	13	47	0.9
		B. typhosus.	No. 13	50	36	14	78	1.5
			Con.	50	4	46	10	0.2
12-28-20	Dog No. 13. 3 hrs. before death.	S. aureus....	No. 13	50	23	27	146	2.9
			Con.	50	37	13	180	3.6
		Pn. I.....	No. 13	50	2	48	2	0.0
			Con.	50	0	50	0	0.0
		B. coli	No. 13	50	36	14	62	1.2
			Con.	50	36	14	49	1.0
		B. typhosus.	No. 13	50	32	18	50	1.0
			Con.	50	19	31	26	0.5
12-28-20	Dog No. 13 Wt. 8½ lbs. Death.	S. aureus....	No. 13	50	27	23	143	2.8
			Con.	50	37	13	180	3.6
		Pn. I.....	No. 13	50	0	50	0	0.0
			Con.	50	0	50	0	0.0
		B. coli	No. 13	50	24	26	58	1.1
			Con.	50	36	16	49	1.0
		B. typhosus.	No. 13	50	38	12	70	1.4
			Con.	50	19	31	26	0.5

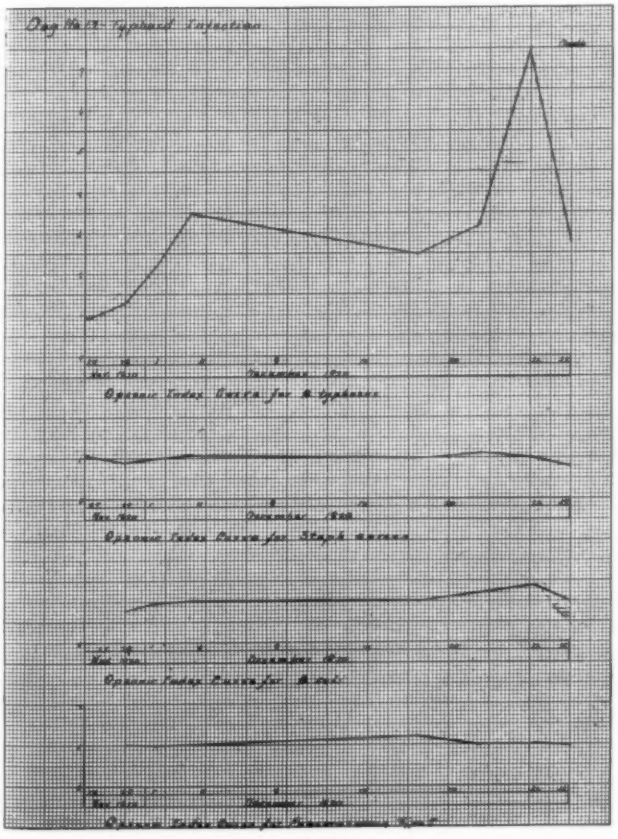

FIG. 4.

Anatomical Diagnosis.—Emaciated; no characteristic typhoid lesions except acute splenic tumor. *B. typhosus* obtained from heart's blood. *B. enteriditis* cultivated from heart's blood and gall bladder.

Kataphylaxis in this animal probably occurred on December 26th when there was a marked decline in the opsonic index for the typhoid bacilli. The decrease, however, was not sufficient to bring the index down to the normal. The phagocytic activity of the blood against *B. typhosus* was at the time of death more than twice as great as that of a normal control. It is possible, too, that the test gives an inadequate measure of the real opsonic strength of the experimental animal's blood, for Harrison[25] has shown that the lytic action of antityphoid serum is so great against the typhoid bacilli as to lower the observed index by dissolving the bacteria before they can be taken up by the leucocytes. That this condition actually existed in this case was demonstrated when the inactivated immune serum gave a higher phagocytic index than when the serum was used unheated. The results of this test are given in the following table:

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic Index
12-18-20	Unhtd. serum.....	B. typho..	No. 13	50	38	12	70	1.4
	Inactvd. serum....		No. 13	50	42	8	124	2.4

That the phagocytic cells in this dog, dying of a typhoid infection, were not functionally impaired is indicated by the following tests.

A quantity of blood was taken in citrate from dog No. 13 and from a control animal. Each lot was washed twice, using 175 volumes of salt solution. Samples from the resulting cells were then separately incubated with *Staph. aureus* and *B. typhosus*. The results are given in the table.

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
12-28-20	30 min. incubation.	S. aureus....	No. 13	50	1	49	4	0.0
			Con.	50	2	48	2	0.0
	30 min. incubation.	B. typhosus.	No. 13	50	32	18	57	1.1
			Con.	50	0	50	0	0.0

Whether the energetic phagocytosis of *B. typhosus* by the washed cells of the immune animal, in the absence of serum, can be interpreted as the action of an immune cell, or whether there was left upon the cell enough highly potent immune serum to opsonize only the specific bacteria is a matter to be decided by more detailed observations. That the last explanation, however, is probably the true one is suggested by Klein's[26] work on the dilution of sera.

A quantity of polymorphonuclear leucocytes were recovered from the urine of dog No. 13 and when incubated with sensitized bacteria were actively phagocytic, as indicated in the following results:

Date	Remarks	Bacteria	*Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
12-28-20	Cells from urine of dog No. 13.	B. typhosus.	No. 8	50	33	17	128	2.6
			Con.	50	23	27	37	0.7

* Animal providing serum.

These findings, then, reveal an animal dying of an infection with its phagocytic mechanism, so far at least as experimental standards can determine, functioning with an efficiency considerably above the normal against the specific organism, without any demonstrable decrease in opsonic activity against the other bacteria used in the test.

Experiment 4.—In this experiment are presented the results of a study of four cases of fatal spontaneous hemolytic streptococcus infection in cats. The epidemic, of which these cases were a part, appeared in a room containing 25 cats, and ran a course so severe that of the whole number only two survived. The onset of the attack was characterized by a nasal discharge and violent sneezing. The cats lost rapidly in weight and died, generally after four or five days. The last 24 hours of the infection were especially severe. The animal was in a state of extreme emaciation and exhaustion, being all the time unable to stand and in the last hours of life responding only to such violent stimulation as a cardiac puncture.

Samples of blood were taken from the heart at intervals indicated in the tables and the usual opsonic determinations made.

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
2-13-21	Cat No. 33. At time of death.	S. aureus....	No. 33	50	43	7	210	4.2
			Con.	50	46	4	188	3.7
		B. coli	No. 33	50	29	21	51	1.0
			Con.	50	27	23	40	0.8
		B. bronchisep.	No. 33	50	50	0	380	7.6
			Con.	50	46	4	285	5.7
		Strep. hemo.	No. 33	50	25	25	101	2.1
			Con.	50	32	18	266	5.3
2-14-21	Cat No. 35. One hr. before death.	S. aureus....	No. 35	50	38	12	161	3.2
			Con.	50	38	12	137	2.7
		B. coli	No. 35	50	27	23	55	1.1
			Con.	50	25	25	62	1.2
		B. bronchisep.	No. 35	50	37	13	166	3.3
			Con.	50	34	16	172	3.4
		B. typhosus.	No. 35	50	36	14	71	1.4
			Con.	50	33	17	86	1.7
2-14-21	Cat No. 36. At time of death.	S. aureus...	No. 36	50	37	13	138	2.7
			Con.	50	38	12	137	2.7
		B. coli	No. 36	50	23	27	58	1.1
			Con.	50	25	25	62	1.2
		B. bronchisep.	No. 36	50	43	7	216	4.3
			Con.	50	34	16	171	3.4
		B. typhosus.	No. 36	50	25	25	55	1.1
			Con.	50	33	12	86	1.7
2-15-21	Cat No. 37.....	S. aureus....	No. 37	50	37	13	102	2.0
			Con.	50	50	0	256	5.1
		B. coli	No. 37	50	48	2	225	4.5
			Con.	50	48	2	216	4.3
		B. bronchisep.	No. 37	50	50	0	294	5.8
			Con.	50	42	8	250	5.0
		Strep hemo.	No. 37	50	25	25	146	2.9
			Con.	50	42	8	213	4.3
2-17-21	Cat No. 37. 28 hours before death.	S. aureus....	No. 37	50	39	11	132	2.6
			Con.	50	46	4	188	3.7
		B. coli	No. 37	50	25	25	45	0.9
			Con.	50	18	32	42	0.8
		B. bronchisep.	No. 37	50	46	4	263	5.2
			Con.	50	48	2	284	5.6
		Strep. hemo.	No. 37	50	34	16	176	3.5
			Con.	50	36	14	266	5.3
2-19-21	Cat No. 37. At time of death.	S. aureus....	No. 37	50	41	9	305	6.1
			Con.	50	46	4	340	6.8
		B. coli	No. 37	50	42	8	216	4.3
			Con.	50	29	21	159	3.2
		B. bronchisep.	No. 37	50	50	0	720	14.4
			Con.	50	50	0	793	15.9
		Strep. hemo.	No. 37	50	32	18	160	3.2
			Con.	50	41	9	201	4.0
2-19-21	Cat No. 37. One hr. after death.	S. aureus....	No. 37	50	46	4	325	6.7
			Con.	50	48	2	327	6.5
		B. coli	No. 37	50	50	0	150	3.0
			Con.	50	45	5	120	2.4
		B. bronchisep.	No. 37	50	50	0	304	6.0
			Con.	50	50	0	297	5.9
		Strep. hemo.	No. 37	50	50	0	201	4.0
			Con.	50	50	0	386	7.7

Anatomical Diagnosis.—All the cats in this experiment presented, in general, the same appearance: Marked emaciation; greenish purulent exudate filling the nasal sinuses and extending over the surfaces of the nasopharynx and down into the trachea as far as the bifurcation. There was a complete absence of any lesions in any other organs. No hemorrhages. *Streptococcus hæmolyticus* in pure culture obtained from the heart's blood of all four animals. This was also the predominating organism in the exudate of the upper respiratory tract.

There is, in general, agreement in the results obtained from these four cases. In all the opsonic index for the specific organism was below normal at the time of death. The opsonic indices for the heterologous bacteria, in each instance, differed from the normal by less than what can properly be regarded as experimental error.

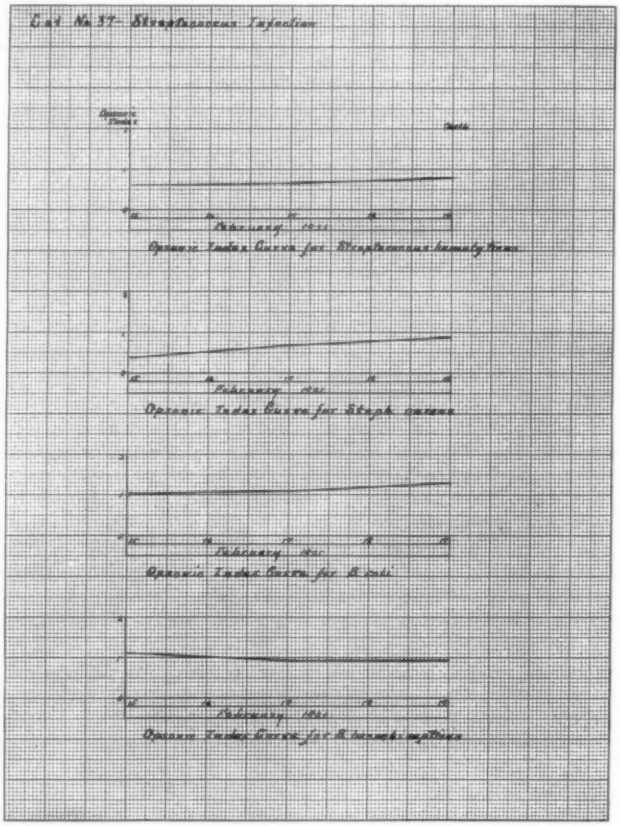
Cat No. 37. Streptococcus Infection
Opsonic Index Curve for Streptococcus hemolyticus
Opsonic Index Curve for Staph. aureus
Opsonic Index Curve for B. coli
Opsonic Index Curve for B. bronchisepticus

Fig. 5.

There was in the study of cat No. 37 one feature of very special significance and interest. This animal when first examined on February 15th already revealed advanced emaciation and weakness. The blood culture on this date showed an abundance of streptococci and a few colonies of *Staph. aureus*. Two days later this latter organism was found abundantly in the blood, but by February 19th it had considerably decreased and had completely disappeared before the cat died on February 20th. Ten cubic centimeters of blood cultured at the time of autopsy failed to develop a single colony of *Staphylococcus*. Streptococci were sufficiently numerous in the blood at all times to be recovered by plating a single loop of blood.

When this cat was placed under observation it was the subject of a double infection and showed indications of being rapidly overcome. That the streptococcus was the dominant etiological factor in the infection seems evident from the fact that the symptoms revealed by this cat were in every way similar to those in animals suffering with a pure streptococcus infection.

Cat No. 37, then, was being overcome by a rapidly fatal infection. It had in some way become secondarily infected with *Staphylococcus aureus*, and this latter organism had become so abundant in the blood stream that there resulted a marked decrease in the opsonins for staphylococci. Yet this enfeebled animal in its losing struggle against a streptococcus infection was able to react against a secondary infection of staphylococcus in a manner so vigorous as to banish completely these bacteria from the body. There was, during this time, a gradual increase in the opsonic index for staphylococcus until at the time of death it was only slightly below the normal. This increase is given in the following table:

		Date		
		Feb. 15	Feb. 17	Feb. 19
Cat No. 37.....	Op. ind.....	0.40	0.70	0.89

It would seem then that the body reacted against the secondary infection in an independent manner, and that recovery from one infection and death from another occurred, by chance, at the same time.

EXPERIMENT 5.—In this experiment an attempt was made to reproduce experimentally the spontaneous infections described in Experiment 4. An adult female cat, No. 38, was injected intravenously with 3 c. c. of an 18-hour culture made directly from the heart's blood of a cat dying of the epidemic streptococcus infection. The cat came down with the characteristic symptoms and died after 72 hours. The extent of emaciation in this animal was not as marked as in the preceding cases.

The routine determinations are recorded in the accompanying table.

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
2-18-21	Cat No. 38. Before injection. Inj. 3 c. c. Strep. hemo.	S. aureus....	No. 38	50	40	10	167	3.3
			Con.	50	46	4	188	3.7
		B. coli	No. 38	50	29	21	50	1.0
			Con.	50	18	32	42	0.8
		B. bronchi-sept.	No. 38	50	47	3	301	6.1
			Con.	50	48	2	284	5.6
		Strep. hemo.	No. 38	50	35	15	306	6.1
			Coh.	50	36	14	266	5.3
2-20-21	Cat No. 38. No injection.	S. aureus....	No. 38	50	39	11	400	8.0
			Con.	50	46	4	341	6.8
		B. coli	No. 38	50	45	5	155	3.1
			Con.	50	28	22	159	3.2
		B. bronchi-sept.	No. 38	50	50	0	850	17.0
			Con.	50	50	0	790	16.0
		Strep. hemo.	No. 38	50	29	21	191	3.8
			Con.	50	42	8	202	4.0
2-21-21	Cat No. 38. Death.	S. aureus....	No. 38	50	40	10	313	6.2
			Con.	50	47	3	328	6.6
		B. coli	No. 38	50	39	11	59	1.2
			Con.	50	45	5	120	2.4
		B. bronchi-sept.	No. 38	50	50	0	320	6.4
			Con.	50	50	0	297	5.9
		Strep. hemo.	No. 38	50	33	17	224	4.5
			Con.	50	20	30	286	7.7

Anatomical Diagnosis.—Slight emaciation; *Streptococcus hamolyticus* in pure culture cultivated from the heart's blood.

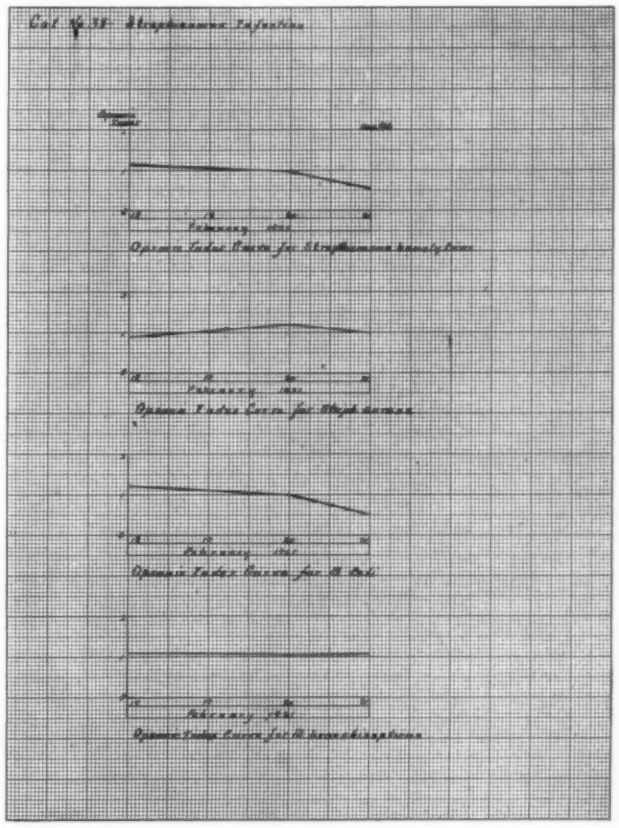

FIG. 6.

The findings in this experiment are throughout similar to the results obtained in the spontaneous streptococci infections.

EXPERIMENT 6.—The dog studied in this experiment had, when first seen on December 27, already developed characteristic symptoms of "distemper." There was a continual nasal discharge associated with frequent sneezing and dyspnea. The animal was emaciated and staggered about when forced to its feet. A small Gram-negative bacillus, probably *Pasteurella canis*, was present in enormous numbers in both the nasal secretion and blood stream. However, on December 30 the dog began to improve and continued to gain in strength until January 12. On this date it suffered an overwhelming and paralyzing collapse. After this, the dog was unable to move and lay in a continual stupor. It died on January 14, in a state of weakness so profound as at once to set it apart from any other animal observed during this investigation.

Full details of the experiment are set forth in the accompanying table.

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
12-27-20	Dog No. 47. Wt. 5.7 kg.	S. aureus....	No. 47	50	33	17	180	3.6
			Con.	50	37	13	199	3.9
		B. coli	No 47	50	45	5	140	2.8
			Con.	50	42	8	102	2.0
		B. bronchi-sept.	No. 47	50	23	27	38	0.7
			Con.	50	26	24	27	0.5

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
1-13-21	Dog No. 47. Wt. 4.8 kg. 13 hours before death.	S. aureus....	No. 47	50	36	14	102	3.2
			Con.	50	44	6	262	4.0
		B. coli	No. 47	50	46	4	146	2.9
			Con.	50	47	3	156	3.1
		B. bronchi-sept.	No. 47	50	48	2	110	2.2
			Con.	50	44	6	62	1.2
		B. typhosus.	No. 47	50	11	39	12	0.2
			Con.	50	10	40	10	0.2
1-14-21	Dog No. 47. Wt. 4.6 kg. Death.	S. aureus.. ..	No. 47	50	41	9	103	3.2
			Con.	50	46	4	202	4.1
		B. coli	No. 47	50	47	3	103	3.2
			Con.	50	47	3	156	3.1
		B. bronchi-sept.	No. 47	50	46	4	112	2.2
			Con.	50	44	6	62	1.2
		B. typhosus.	No. 47	50	5	45	6	0.1
			Con.	50	10	40	10	0.2

Anatomical Diagnosis.—Marked emaciation; purulent exudate in nasal cavity and pharynx; extensive broncho-pneumonia. *Pasteurella canis* (P) cultivated from blood and from nasopharyngeal exudate.

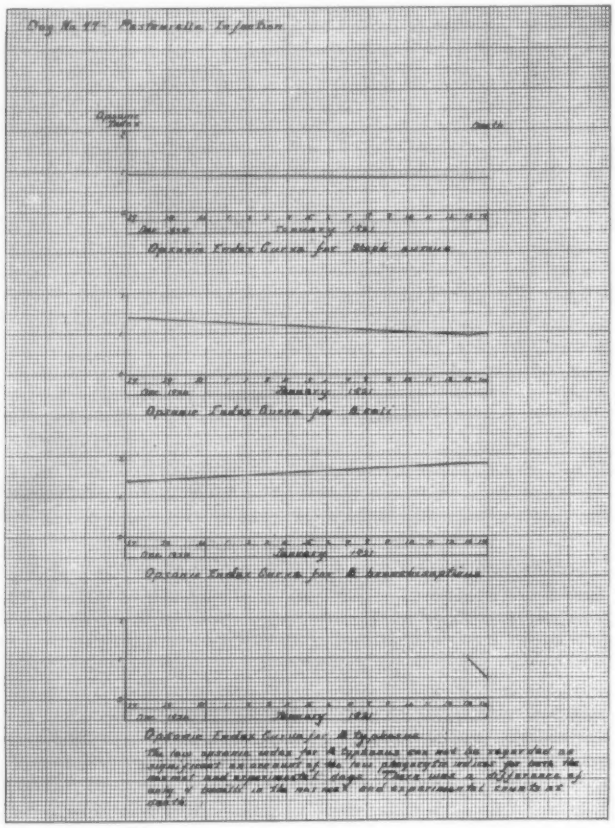
Dog No. 47 Pasteurella Infection
Opsonic Index Curve for Staph. aureus
Opsonic Index Curve for B. coli
Opsonic Index Curve for B. bronchisepticus
Opsonic Index Curve for B. typhosus
The two opsonic units for B. typhosus are not to be regarded as representing an account of the true phagocytic value for both the normal and experimental dogs. There was a difference of unity, if counted in the normal and experimental counts as such.

FIG. 7.

It does not seem possible that any animals could attain a more defenseless condition than this dog presented during the last 36 hours of its life, and yet throughout this period there was no discoverable rupture in its phagocytic defense.

DISCUSSION

Throughout this investigation it has been the custom in each enumeration to determine not only the total number of bacteria taken up by 50 neutrophile leucocytes, but also to record the number of these cells actually taking part in the engulfment. A survey of these records reveals in general a parallelism between the variations in the phagocytic indices and fluctuations in the percentage of phagocyting cells. Commonly a high phagocytic index is associated with a large percentage of phagocyting leucocytes, while a low index generally is obtained in preparations with few ingesting cells. However, this is not uniformly true and of the two methods for determining the degree of phagocytic activity it would appear from this research that the technique of counting the number of organisms ingested is the more delicate and reliable. It has frequently happened that the experimental animal revealed the same percentage of phagocyting cells as the control but yet exhibited the utmost variation in the total number of bacteria ingested.

There is always a question whether the opsonic results obtained by incubating samples of cells and serum with indifferent suspensions of bacteria can be accepted as indicating even approximately the conditions which prevail in the animal body. It is, of course, at once recognized that organisms grown on artificial media may differ fundamentally, in their reaction to living cells, from bacteria which develop within the body. Bordet[25] has shown, for example, that even when the most virulent organisms are injected into a susceptible animal, there is an initial phagocytosis. This he explains as a selective process. Supposing that any culture, no matter how virulent, contains numbers of feeble and weakly aggressive individuals and these the phagocytes at once select and devour. The remaining highly selected bacteria, then, in some cases, extend the infection without any phagocytic interference. Zinsser[26] likewise has pointed out that it is possible to obtain agglutination of "culture" pallida but quite impossible to do so when the spirochetes are taken directly from a lesion. A certain reasonableness therefore attaches itself to the objection that with phagocytic determinations *in vitro* one may be dealing largely with the enfeebled non-pathogenic fraction of the cultures examined and that the findings are worthless as an index of vital phagocytic defense. It cannot be denied, however, that opsonic determinations possess some significance and diagnostic value. It is possible that such observations in a specific case may be of uncertain value, but when extended over a large series, representing a wide variety in species and conditions, a uniform result gives ample justification for conservative deductions.

Perhaps the most striking feature of this investigation has been the unfailing effectiveness of phagocytic activity against the non-specific bacteria. In not one instance has there been a general decline in the opsonic indices for microorganisms not concerned in the primary infection. This has been uniformly true even in cases of extended infection, associated during the last days with the most destructive emaciation and with what appeared to be complete collapse of the animal's vital defensive mechanism.

It appears certain that the percentage of infected animals dying because of a collapse in the phagocytic defense is very

much smaller than is commonly supposed. Indeed, it is quite often as inaccurate and meaningless to say that an infected animal is overcome on account of a rupture in its phagocytic defense as it would be to contend that a man was run down by a train because of a break in his running technique, although at the time of the disaster he was exhibiting a speed beyond anything he had ever developed before in his lifetime.

CONCLUSIONS

The results obtained in this investigation would seem to warrant the following conclusions:

1. No decrease in phagocytic activity against any bacteria not concerned in the primary infection has been demonstrated even in the late stages of fatal infection. This would suggest that terminal infections do not arise primarily as the result of a rupture in the phagocytic defense.

2. A subnormal opsonic index for the specific infecting organism is not an invariable phenomenon in fatal infections.

3. Death in fatal infections does not always occur at the period of lowest opsonic activity.

In closing this paper I wish to thank Dr. W. G. MacCallum for his courtesy and assistance, and I desire to acknowledge a very especial debt of gratitude to Dr. S. Bayne-Jones for the substantial contributions he has made to the development of this problem.

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THE CHEMICAL DYNAMICS OF MUSCLE[1]

By F. GOWLAND HOPKINS

In the previous lecture a few facts and some suggestions concerning oxidations in living tissues were discussed wholly from the standpoint of the chemical reactions involved. The biochemist must, however, recognize that the chemical reactions which it is his business to study occur in very peculiar apparatus. The structure of this apparatus is a factor—perhaps the most important factor—leading to that organization of chemical events which is the fundamental characteristic of any living unit. When the structure of a tissue is destroyed, oxidative processes may continue in the disintegrated material; but, as Warburg has remarked, they can then yield heat alone.

Specialization in the tissue-structure or apparatus may so influence the organization of chemical reactions—even of those which are common to all living tissues—that the final result of their progress, and the nature of the associated energy discharges, may be profoundly modified from case to case. The biochemist, while always concerned with the precise nature of the reactions themselves, must go further and study so far as he can their progress in the living tissue itself.

[1] Lecture II of the Herter Series delivered before The Johns Hopkins University on April 13, 1921.

That this is not an impossible task is illustrated, I think, by chemical studies of striated muscle. Aided by thermodynamic studies they have already yielded knowledge, which, though very far indeed from affording a complete understanding of the relations which exist between the metabolism of muscle and its mechanical activities, is more than sufficient to show that experimental attacks upon such problems are justified. Viewed indeed from a purely chemical standpoint the dynamic events in this highly specialized tissue appear to be relatively simple, though the means which adjust them to a particular end are doubtless complex enough. Even in the latter connection, however, experimental studies are at the moment yielding interesting data of an unexpected kind, especially perhaps with regard to the functions of oxygen.

I ventured in my previous lecture to express disbelief in theories which refer the chemical manifestations of life to the properties of a hypothetical entity—the biogen or living molecule.

It was in connection with muscle when in 1867 Hermann put forth his *inogen hypothesis* that in an elementary form such theories arose. In my belief a fair consideration of the

facts now known about the muscle is sufficient to prove them wrong.

You will of course remember that in Hermann's view the contraction of muscle follows upon the sudden, almost explosive, breakdown of a complex substance leading to the appearance of lactic acid and carbon dioxide. This unstable stuff is inogen, the source of energy. At first, influenced by his belief that the processes of Rigor were in essence similar to those of a Contraction, Hermann thought that the protein myosin, which separates in a gelatinous form during rigor, was part of the inogen. It is sufficient, however, to remember that the essence of Hermann's teaching was that the production of carbon dioxide in muscle depends not upon the direct and contemporary oxidation of relatively simple compounds, but upon the transference of oxygen, previously combined in a complex molecule, from less stable to more stable relations. Thus arose that conception of intramolecular oxygen which has given form to many biological speculations. Up to near the end of the last century the evidence seemed to justify it fully enough. Spallanzani, who seldom was in error, had shown even a century earlier that living tissues could long survive and continue to yield carbonic acid without any supply of oxygen except such as had been previously available. Spallanzani's statements were in later years fully confirmed by others, and Hermann made the evidence rather more definite in the case which especially interests us by showing that no trace of oxygen could be pumped out of a muscle which, nevertheless, could actively contract without an external oxygen supply, and at the same time produce carbon dioxide. Hermann's consequent views related in particular to the case of muscle, but as you well know, Pflüger later on (with other speculations as to the respective nature of dead and living protein) taught that all tissue respiration is based upon the activity of intramolecular oxygen.

Such views were almost unchallenged for 30 years, and since I am now to speak of work done in Cambridge, England, it is of interest to point out that, in a certain form at least, they satisfied the critical mind of him who founded this Cambridge school, as any who read the last edition of his famous text-book will discover. This edition had not long left the press, however, when one of the last of the brilliant group of workers directly inspired by Michael Foster began some work which was to change the whole outlook.

In 1898 H. M. Fletcher[2] published an account of an observation which, like many other observations of great importance, was in a sense of simple nature. The technique which made it reliable was not simple, however, and the value of the observation arose from its quantitative nature. Properly estimated it will in my opinion be found to have wholly removed the foundation of a belief which, as we have seen, had long dominated animal physiology. Excised surviving muscle gives out, it is true, a steady stream of carbon dioxide. It is clear, however, and it is an assumption, implicit if not explicit, in all that has been attributed to Hermann's inogen, that if this CO_2 arises from this hypothetical unstable source of energy, the amount should increase when the muscle passes from rest to activity. The breakdown of the inogen, which is *ex hypothesi* the source of energy, must surely be increased when the liberation of energy is increased. Therefore, since the inogen is also the source of the CO_2, a muscle, though wholly deprived of oxygen, must give out more CO_2 when it contracts than when it is resting. Fletcher showed that it did not.

The fact is fundamental. So long as a muscle remains in any sense normal, prolonged stimulation under anaërobic conditions fails to produce any effect upon its small but steady output of CO_2. Very different, as Fletcher further showed, is the case where a contemporary supply of oxygen is available. The output of CO_2 during rest is then greater and this output is at once increased when work is done. This difference between the chemical response to stimulation under anaërobic and aërobic conditions, respectively, is again fundamental. There is no evidence for the breakdown of a previously constructed inogen, but there is clear evidence that an increase in contemporary oxidations is associated with contraction.

But what of that relatively small but steady evolution of CO_2 which does after all occur anaërobically whether the muscle be active or at rest? It may be stated at once that it is not a product of metabolism at all. It is not physiological. When indeed we attempt later to picture the cycle of normal events, we may frankly forget it. It is merely carbon dioxide liberated from alkaline carbonates in the muscle as a result of the steadily increasing acidity which, as we are to see, is characteristic of the anaërobic conditions. To the best of my belief it was H. M. Vernon[3] who first suggested the possibility of such an origin: Hill afterwards referred to it, and later Fletcher[4] settled the matter by experiment.

Fletcher's early work provided further facts of significance. He fully confirmed the historical statements of Humboldt and later observations of Joteyko by showing that exposure to pure oxygen greatly delayed the onset of fatigue and of rigor mortis in excised muscle, but he went further and showed that a muscle which has lost the greater part of its irritability and is near to stiffening may be restored if exposed to pure oxygen.[5] From the facts which he made available Fletcher drew a conclusion which foreshadows an essential part of the story I hope to unfold. "It is suggested" he wrote in 1902, "that the hastening of rigor mortis and fatigue in a muscle from which oxygen is withheld is due to increased accumulation, under circumstances of deficient oxidation of the metabolic products within the muscle which are the potential precursors of carbonic acid"; while in the presence of oxygen "the dissociation processes alike of resting and active muscle, advancing slowly in the former, rapidly in the latter, result in the formation of free CO_2."

Since from the days of Berzelius lactic acid had been known as a constituent of muscle and many observers led by DuBois-Reymond had associated its production with activity and rigor,

[2] H. M. Fletcher: Jour. Physiol., 1898, XXIII, 10.

[3] Vernon: Science Progress.

[4] Fletcher and Brown: Jour. Physiol., 1914, XLVIII, 177.

[5] Fletcher: *Ib.*, 1902, XXVIII, 476.

Fletcher was of course alive to this probability that this acid is among those precursors of CO_2 which accumulates anaërobically, but he avoided at this time any dogmatic statement on the matter. The state of the literature as it then stood fully justified such caution.

Not long before the statement had been made by more than one observer that as a matter of fact there is no more lactic acid in a muscle in full rigor than in one perfectly fresh. Older views—which had never been supported by really quantitative work—were traversed and the significance of lactic acid in muscle had become entirely obscure.

A quantitative study was clearly necessary for further progress and in 1907 it was my privilege to join forces with Fletcher in an attack upon the problem.[6] Although the results of our somewhat laborious research have failed to affect the teaching of some text-books we have the satisfaction of knowing that they have been the acknowledged point of departure for recent important studies.

We found that the confusion in the literature as to the quantitative relations of lactic acid in muscle were wholly due to faulty technique in dealing with the tissue itself. When the muscle is disintegrated as a preliminary to extraction for analytical purposes, the existing equilibrium is entirely upset. Interacting factors are brought into abnormal relations and the processes of change are greatly accelerated. A fresh muscle had been supposed to contain as much acid as one in rigor simply because it had been produced in the former by the treatment which had preceded estimation. The biochemist had not sufficiently remembered the instability of his material. Fletcher and I, however, found it quite easy by means of a simple method, not only to avoid starting the changes which led to the formation of lactic acid, but to arrest them at any point during their progress and thus establish their time relations.

We were able to show that the accumulation of lactic acid in muscle occurs only in the conditions of anaërobiosis. With a proper oxygen supply it fails to accumulate at all, though this is not to say that it fails to be produced. Its formation anaërobically in a quiescent muscle shows no irregular relations: it is not, for instance, delayed until rigor appears, or is near. The accumulation starts from zero or from the minimal quantity present in fresh muscle and continues at a linear rate, equal amounts being produced in successive units of time. The reaction responsible for its appearance has a normal chemical temperature coefficient, at least between such temperatures as 10 to 20° C. At higher temperatures the effects of changes in the tissue structure become of influence and the process is accelerated so that at 40° C. there is a very rapid rise to that "acid maximum" which Kanke described long ago. At still higher temperatures and especially near 100° C. the process, like other biological processes, is arrested.

Stimulation increases the rate of production as should be expected. We have indeed every reason to believe, and I shall later give recent evidence to support the belief, that in muscle the chemical processes of activity differ quantitatively only, and in no way qualitatively, from those that proceed during quiescence. If a muscle be tetanized to complete fatigue Fletcher and I found that the lactic acid produced always stopped short of that maximum which is found in rigor mortis or produced rapidly at 40° C. In the fatigue of tetanus the amount is about half that of rigor (say 0.2 per cent as against 0.4).

Roughly to be classed as chemical stimuli are the effects of certain volatile organic substances, such as chloroform, toluol and others. When a muscle is exposed to the vapour of these a maximum yield of lactic acid is soon reached and a form of rigor is established, possibly because as solvents of lipoids these substances profoundly affect permeability relations within the muscle structure.

The next point established by Fletcher and myself is of fundamental importance. If a muscle which, by exposure to anaërobic conditions, has accumulated lactic acid, be placed in oxygen, the acid is removed. The occurrence of this removal under the influence of oxygen is significant in all that follows. It has been fully confirmed by the later work of Parnas and Meyerhof.

The experimental findings so far discussed have removed all basis from the theory of intramolecular oxygen, and have shown that an excised muscle is, at any rate, quite able to obtain free energy from its stores of potential energy without the aid of oxygen. On the other hand, while anaërobic existence, passive or active, inevitably involves an accumulation of lactic acid in the muscle, subsequent exposure to oxygen effects its removal. If, however, oxygen be available from the first, it must be remembered that the acid does not accumulate at all.

There are, therefore, at this stage of the enquiry perhaps two possibilities to consider. The muscle when forced to dispense with oxygen may be supposed to derive its energy from potential stores by chemical steps differing entirely from those which occur under physiological conditions. If so, lactic acid may be an abnormal product playing no part in normal events. On this view the anaërobic events are merely vicarious. But another possibility is that the normal cycle of events consists always of two phases separated in time: the first anaërobic during which lactic acid is formed, the second aërobic during which it is removed. Now the conception of a respiratory sequence in which anaërobic events precede and prepare the way for final oxidations has long been familiar to the minds of plant physiologists. Pfeffer, for instance, long ago spoke of the former as processes of intramolecular respiration, and looked upon them as necessary antecedents to subsequent oxidations. It is such a view that I propose to develop in connection with muscle, and I shall now proceed to give you further evidence in its favor.

But the facts won in the study of muscle indicate that the influence of oxygen has in the case of animal tissues more complicated relations than those usually postulated for the case of plant tissues: relations which seem to me to have extraordinary interest.

[6] Fletcher and Hopkins: Jour. Physiol., 1907, XXXV, 247.

At the close of our work upon lactic acid Fletcher and I were convinced that the influence of oxygen upon the activities of muscle is exerted after, rather than before or during, the actual act of contraction, and we were supported in this view by an observation previously made by Danilewsky [7] who found that heat is given out by a contracting muscle after the actual contraction is over: but the ordinary methods of chemical analysis seemed hardly capable of affording a final proof of this sequence. Clear evidence concerning the time relations of events could be best got from the study of a single contraction, a task impossible for the chemist.

At this stage, however, the Cambridge School of Physiology gained an unusual advantage by recruiting, in the person of A. V. Hill, one who is at once an accomplished mathematician and a skilful experimentalist. Hill's qualifications have enabled him to advance very greatly the thermodynamics of muscle and to supply highly essential links in the chain of evidence which has led to the standpoint I am to advocate.

His studies have been made partly on the lines of microcalorimetry, but also, and more particularly, by the use of thermoelectric methods which he has greatly refined. A fundamental observation made early in Hill's researches puts upon a firm experimental basis the conclusion of Danilewsky already quoted, namely that the heat given out by an active muscle long outlasts the contraction itself.[8] But Hill's further time analysis of the thermal phenomena is so important to my thesis that it should be very fully grasped at least in its essentials. The essentials are these. Directly associated with a contraction is a rapid evolution of heat, which occurs both under anaërobic and aërobic conditions, and is, moreover, alike in its time relations and other characters, wholly unaffected by the presence or absence of oxygen.

Oxygen, however, has a noteworthy effect upon the sequel. In its absence the heat evolution just mentioned is quickly over, and is the only thermic change associated with the act of contraction. In its presence there is a further evolution of heat subsequent to, and long outlasting, the act. These facts are clearly of great significance. The process which comes first must be anaërobic: that which comes second can scarcely fail to involve an oxidation. We have here a proof of the suspected sequence of events, and data which strongly suggest that at the moment of contraction, even under conditions made normal by the available supply of oxygen, something is produced anaërobically upon which oxygen subsequently acts. The chemical facts already discussed leave but little doubt that this substance is lactic acid, and evidence will be immediately given to show that the formation of the acid is as a matter of fact intimately associated with the process which gives rise to the early anaërobic heat production. Later on we shall see that there are abundant reasons for associating the second oxidative heat production with the occurrence of its removal.

[7] Danilewsky, Pflügers Arch., 1889, XLV, 353.

[8] A. V. Hill: Jour. Physiol., 1910, XL, 389; with series of subsequent papers in the same Journal.

Already we may speak, at least provisionally, of two phases in the muscular act, separated in time—an anaërobic phase of activity, or *fatigue phase*, and an aërobic phase, or *recovery phase*. It is convenient to make this distinction now. Further justification for it is inherent in all that follows.

The recovery phase must not be confused with the relaxation of the muscle, which, like the contraction proper, is primarily independent of oxygen and is included in the first phase. I shall deal first with this phase of activity.

Early in his studies Hill demonstrated a fact which yields an important point in the evidence. Between the mechanical tension which appears in the muscle and the production of heat, there is always a direct proportionality. Alike in the steady anaërobic metabolism of the quiescent muscle, or during stimulation, or in anaërobic various forms of rigor however rapidly induced, the appearance of a given quantity of acid is associated with the evolution of a proportional quantity of heat. A specially accurate quantitative proof of this was given by the work of R. A. Peters [9] at Cambridge. Using admirable technique he showed that while the indirect stimulation of muscle to fatigue involved the evolution of 0.9 cal. per gram of tissue, the subsequent induction in the same muscles of rigor by chloroform added 0.89 cal. per gram. When rigor was directly induced in fresh muscle the heat production amounted almost exactly to the sum of the above, and double the heat of fatigue, namely 1.70 cals. Now Fletcher and I had shown, as already mentioned, that in rigor the average production of lactic acid is about double the production in fatigue, and this Peters confirmed. Each gram of acid when it appears in muscle is accompanied by the appearance of about 450 calories, and the constancy of this ratio under such different conditions shows that acid and heat owe their origin to one and the same process. Equally important in the development of the views I am putting before you is the indication supplied by such results that the events which give rise to lactic acid formation during normal activity are in no sense of a different order from those which are responsible for the appearance of the acid during the anaërobic metabolism of rest, or in rigor however produced or accelerated.

These results have been in all essentials amply confirmed by the very recent work of Meyerhof,[10] to which in other connections I shall have to make frequent reference.

His experiments have an advantage over the earlier ones in that the estimations of heat and lactic acid were made upon the same set of muscles, and the comparison was direct. At the same time it must be remembered that only micro-methods could be used for such a purpose. Meyerhof found, as the result of change in conditions such as the temperature at which the muscles are stimulated, certain variations in the heat evolved per unit of lactic acid produced, but proved that the relation was essentially of the same order as that assumed by Hill and Peters. With regard to the effect of temperature Hill has very recently shown that in the response to a single

[9] Peters: Jour. Physiol., 1913, XLVII, 243.

[10] Otto Meyerhof: Pflügers Arch., 1919, CLXXV, 20 and 88; *Ib.*, 1920, CLXXXII, 232 and 284.

shock the relation is the same at all temperatures. In prolonged stimulation it varies. For average conditions we shall be close to the mark if we take, with Meyerhof, 400 small calories as the heat production which is normally associated with the formation of 1 gm. of lactic acid.

Lactic acid is related in a similar quantitative way with mechanical changes in the muscle. To get clear on this point we must realize certain properties of the muscle as a machine which have been brought fully to light by the investigations of Hill. Earlier experiments had shown that the actual work performed by a contracting muscle bears very variable and apparently accidental relations to the heat evolved. The case is different, however, if we deal with the primary mechanical event which follows immediately upon stimulation and precedes any performance of work. This is a sudden increase of tension in the fibres which can be accurately measured. It means that the elasticity of the fibres has suddenly changed, and its occurrence implies a sudden conversion of chemical potential energy into mechanical potential energy. The subsequent developments in the muscle depend upon the circumstances in which it is placed. If it be free to contract, then part of the energy of the new tensile stress will appear as work and part, of course, as heat. If the muscle after stimulation cannot contract, the whole appears as heat, Hill having established the important fact that the heat evolution is then always proportional to the amount of tension previously established in the muscle. Much, I may say here, of what is quantitative in our present knowledge is due to Hill's recognition of the importance of studying the dynamics of the isometric contraction. Such studies have yielded proof of the relation just mentioned.

Since the mechanical potential energy established in the isometric muscle by stimulation degrades into a proportionate amount of heat, and this heat has been shown to be proportionate under all circumstances to the lactic acid produced, it follows that the original mechanical potential is proportionate to acid production. This Meyerhof has directly shown by estimating the integrative effects of a series of isometric contractions carried on to the point of fatigue. He found that the total mechanical effect is in general proportionate to the total acid formed, the absolute value of each varying with such factors as the nutrition condition of the animals used.

The relations just discussed compel the belief that acid production, the development of tension, and the heat which ultimately leaves the muscle, are all essentially due to one event and that a chemical change following immediately upon excitation. Here clearly we reach a standpoint based upon quantitative data of the utmost importance for the understanding of the dynamics of muscle.

Our next desire will naturally be to discover the nature of the primary chemical reaction concerned. We need then first to know what is the precursor of the lactic acid. All the probabilities would point to carbohydrate as the ultimate, if not the immediate, source of the acid, but the experimental evidence supposed to bear on the matter seemed for a long time hopelessly confused. It is not necessary to enter into the history of controversy on the point. Much of it has been due to the fact that the data upon which arguments have been based were yielded by unsatisfactory methods and so were contradictory and without quantitative value. Recent work and especially that of Parnas and Meyerhof shows at least that whenever lactic acid appears in muscle an equivalent quantity of carbohydrate disappears. These observers used micro-methods for their estimations, though their results were carefully controlled. In my laboratory we have recently repeated the observations using similar methods with larger quantities of material. We have been impressed by the exactness with which the amount of lactic acid formed under any circumstances corresponds with the amount of carbohydrate simultaneously disappearing.

Seeking detail as to the exact nature of the precursor we may pass at once to the results of the patient investigation of the Frankfort School. Doubtless having in view the classical discovery of Harden and Young, who showed that in the chemical breakdown of sugar by yeast juice the synthetic formation of a phosphoric ester of hexose is an invariable event, and also being aware of certain data in the literature which suggested that during the activity of muscle inorganic phosphates may be increased at the expense of phosphates organically bound, Embden and his colleagues, Laquer and others, set themselves to look for hexose phosphate in muscle.

The final outcome of a patient and laborious investigation was the proof that this hexose phosphate does undoubtedly exist in muscle and shares in the processes of change.[11] Moreover, muscle-extracts and other tissue-extracts, while quite incapable of converting free sugar into lactic acid, break down the hexose phosphate in such a way as to yield both lactic and phosphoric acids. The view may be taken, and if true, it is one of great interest, that the association with phosphoric acid in some way makes easier that intramolecular shift which is involved in the production of lactic acid from dextrose. We can hardly doubt, therefore, that between the glycogen and the lactic acid of muscular metabolism this combination of sugar with phosphoric acid is an intermediary. It is probably the immediate precursor of the latter and is in any case a precursor at some stage in the processes of production. Its properties justify the title "lactacidogen" which Embden has applied to it.

In their more general aspects the occurrences of the active phase of a muscle act may be pictured as follows: As the result of stimulation, sugar originally derived from the glycogen stores but situated in some position of advantage in the muscle structure and almost certainly combined there with phosphoric acid suffers a sudden breakdown yielding lactic acid. As a result of this chemical event the muscle elements suffer a marked change in respect of their elastic properties and this leads to a development of mechanical tension within them proportionate in amount to the acid produced. This tension may then be converted into work, but in such conditions as those which exist when a muscle contracts isometri-

[11] Embden and Laquer: Zeitsch. f. physiol. Chem., 1916, XCVIII, 181; 1921, CXIII, 1.

cally it is wholly degraded into heat. This heat, though by no means to be thought of as identical with the heat of the initial chemical reaction, is always proportionate to the extent of that reaction, and so also to the lactic acid produced. So long as the events of the next phase—the recovery phase—are for any reason in abeyance, the acid accumulates in the muscle. But these events, in which the influence of oxygen is dominant, must be now considered.

The recovery period is most easily pictured when conditions sharply demarcate it in time, as when a muscle properly supplied with oxygen is engaged in a series of single contractions. It then intervenes between relaxation and the following contraction. But the evidence shows that the chemical processes involved in it, so long as oxygen is fully available, continuously play their part in the chemical equilibrium of muscle whether quiescent or active.

The facts already discussed have shown that the main event during recovery is the disappearance of lactic acid. But the precise mechanism of its removal is a matter vital to our understanding of the dynamics of muscle. If, for instance, we endeavour to take the simplest view of the situation and in analogy with what has been taught concerning plant respiration conceive that the sequence of events is merely that of acid production during activity, followed by complete combustion of the acid, during recovery, we meet at once an inherent difficulty which has prevented many in the past from recognizing in lactic acid a normal product of activity.

The demands of a working mechanism such as muscle are somewhat different from those of more quiescent plant tissues. It calls not only for a supply of available free energy but for the delivery of this at the right moment.

Now in the breakdown of carbohydrate to yield lactic acid a very small fraction, perhaps one-fortieth, of the chemical potential of the former is made available as free energy. The rest would be set free during the subsequent combustion, but as the act of contraction has *ex hypothesi* preceded this, the natural conclusion would be that the mechanical activity receives no support from oxidations, which then would provide heat alone.

Under such circumstances the consumption of material necessary to yield a given amount of work would be out of all proportion greater than what is known to occur in the normal intact animal.

If, then, the splitting of carbohydrate into lactic acid as a source of energy for the contraction be no abnormal process, vastly uneconomical, to which the muscle is merely driven by deprivation of oxygen; if the sequence of events we are picturing be real, then in some way, energy must somehow be made available for activity during what we have called the recovery phase. Recent research has made it clear that there is a real post-contractile return of material and energy to the system of the muscle. The facts are of great interest and would seem to bear not alone upon the dynamics of muscle but no less perhaps upon the nature of the relations between oxygen and living processes in general.

During our work upon lactic acid Fletcher and I obtained an experimental result which seemed to us significant. We submitted muscles to processes of alternating fatigue and recovery repeated many times. As during each period of recovery lactic acid left the muscles it is clear that the treatment referred to if it involved complete oxidation must ultimately have made a heavy drain upon the sources of the acid. Organs so treated might be expected to show sign of consequent deficiency in these. Yet when they were warmed to 40° C., so as to obtain from them Ranke's "heat maximum" it was found that they yielded just as much lactic acid as perfectly fresh unfatigued muscles. Though we tried not to dogmatise upon the matter, we thought that here was some justification for believing that the acid was, during the recovery periods, not burnt away, but perhaps rebuilt, not, of course, with the oxygen into an inogen, but into whatever substance had first yielded the acid anaërobically. It was afterwards shown, however, by Laquer that a simpler explanation for the facts is available. The production of lactic acid of muscle is a self-regulated reaction stopping short when a certain maximum is reached. If, therefore, the original supply of precursor is relatively large, we need not expect that its partial exhaustion will appreciably reduce the final maximum. There are certain considerations to suggest that the experimental result described is not entirely to be explained in this way; but I need not dwell upon them because there is now other and more direct evidence to show that the processes of recovery reverse the processes of fatigue.

Quite early in his researches Hill measured the heat given out in the period which follows a single muscular contraction, and found this so-called *heat of recovery* to be quantitatively of the same order as the *heat of contraction*. To this result he applied the following argument: The lactic acid produced in contraction disappears during recovery and since the heat associated with the formation is known to be about 400 cals. per gram, while the heat of recovery is about the same. only some 400 cals. can be evolved during the removal of 1 gm. But the total heat of combustion of 1 gram of lactic acid is something like 3700 cals. It is impossible, therefore, that more than a fraction of the acid which is removed during recovery can be actually burnt. Most of it must be removed in some other way; as Hill thought, by reconstruction into a substance of higher potential energy.

But before the argument can be completed, more precise and direct information is wanted as to how much material is actually oxidised in the recovery phase. This is not necessarily measured by the heat given out. It can be determined, however, by measuring the amount of oxygen consumed, especially if at the same time we get to know the respiratory quotient. Such a determination was first made by Parnas[12] in the course of work begun at Cambridge. Parnas found first of all that the oxygen consumption of an excised muscle, resting, but previously fatigued and therefore loaded with lactic acid, was definitely greater than that of a fresh resting muscle containing at most a minute quantity of acid. His results seemed

[12] Parnas: Centrlb. f. Physiol., 1915, XXX, 1.

to show that this excess in the oxygen consumed corresponded with some exactness to the combustion requirements of the amount of lactic acid known to have disappeared from the fatigued muscle by the end of the experiment. But he found also, and here he demonstrated a point of great importance, that the heat actually evolved was considerably less than what this combustion would produce; the former being indeed only half the latter. Parnas drew from his results the conclusion that while the lactic acid was not resynthetised, but wholly burnt, the energy of its combustion was not all lost to the muscle. On the contrary, a moiety of this energy is by some unknown means employed in restoring potential to the contractile system.

You will not deny that this return of energy to the physical mechanism under the influence of oxygen is a significant event. Its occurrence has been confirmed by the later work of Meyerhof.

In a series of papers which have appeared during the last year this author, who had previously identified himself with productive studies of general tissue-respiration, has made important contributions to the subject of this lecture. Much of his work constitutes a confirmation of that of Fletcher and myself, as also that of Hill and Peters; though, especially in connection with the effect of conditions on lactic acid formation, he has added precision to details. But he has also added facts, and especially one fact of great importance.

Meyerhof repeated Parnas's experiments on the oxygen consumption of the recovery period, estimating directly in the the lactic acid which disappears. The conclusion that part of the energy arising from the oxidation is retained in the muscle was confirmed, but Meyerhof's results differed quantitatively from those of Parnas, and the difference modifies the interpretation of the facts. Meyerhof agrees with Hill in finding that the observed heat of recovery is of the same order of magnitude as the heat of contraction, though his results suggest that the former is somewhat the greater, in the ratio, say, of five to four. He finds with Parnas that this value is considerably less than what would correspond with the lactic acid (or carbohydrate) burnt, but the figures he obtained for oxygen consumption indicate that not the whole but no more than one-third to one-fourth of the lactic acid is actually burnt; the remainder disappears without oxidation. This was Hill's view. But Meyerhof has gone beyond previous observers by showing what this disappearance means. The balance of the acid he finds is actually reconverted into glycogen!

E. J. Lesser,[13] several years ago, showed that the glycogen of intact frogs was greatly reduced as the result of a comparatively brief deprivation of oxygen, and that when the animals were returned to normal respiratory conditions, without receiving food, the total glycogen returned in part to its original value. This might possibly indicate a resynthesis from lactic acid formed during the anoxybiosis. But the process in the whole animal might have been quite indirect, and involve other organs than the muscles. Meyerhof on the other hand, by simultaneous estimations of lactic acid and glycogen in excised muscles, before and after their recovery in oxygen from previous fatigue, has shown directly that during the process the one is actually reconverted into the other.

[13] Zeitschr. f. Biol., LI, 287.

Meyerhof's results have been fully confirmed in my laboratory by the conjoint work of Miss D. Foster and Miss Moyle.

Energy, it would seem, is returned to the muscle along two lines; one involving a restoration of chemical potential, and the other the restoration of potential to a physical system. Each event has its own importance in the return to the *status quo ante*.

From the chemical standpoint the equilibrium changes involved during activity and recovery resolve themselves ultimately into the simple reversible equation:

$$\text{Glycogen} \rightleftharpoons \text{Lactic acid.}$$

So long as anaërobic conditions prevail the production of lactic acid is an irreversible process; under the influence of oxygen it becomes reversible, though at the cost of the oxidation of a part (say one-fourth) of the reacting materials.

We may provisionally put the relations just discussed into quantitative form with the data of Hill and Meyerhof as a basis. Calculated as for one gram of muscle the average of Meyerhof's results gives for the heat of contraction 0.75 cals.; for that of recovery 1.0 cal.; and for the heat of the lactic acid (or carbohydrate) actually burnt during recovery 1.75 cals. If we calculate in round figures the calories corresponding to 1 gram of lactic acid either produced or removed we get the data in a convenient form:

Heat of anaërobic contraction	Heat of recovery in oxygen	Total heat of activity in oxygen	Heat of combustion of materials burnt
400 cals.	500 cals.	900 cals.	900 cals.

This is a balance sheet which any auditor would pass as satisfactory. The heat lost as the result of an exothermic change during the fatigue phase (400 cals.) is balanced by the absorption of an equal amount of heat due to an endothermic change during the recovery phase. On the other hand, the heat of combustion of materials actually shown to have been oxidised exactly provides the total loss of energy (900 cals.) during the whole cycle of change (400 + 500 cals.). As a result of the recovery processes, therefore, the muscular system is restored to the condition which existed before activity, except for the fact that, in the disappearance of part of its glycogen store, it has lost chemical potential energy equivalent to the energy it has expended.

There is temptation at this stage to make some attempt towards bringing the chemical facts and the related thermal data which have been discussed into some definite picture of the whole mechanism of contraction. To do so would involve entering a field which is still largely speculative, and any such attempt to be worth while would call for much time. It will be well, therefore, if I continue to confine my remarks within the limits indicated by the title of the lecture and deal with chemical aspects of the subject alone.

I would like, however, just to make the claim that, properly estimated, the evidence available should be held sufficient to

make it probable that at some point in the cycle of muscular activity the appearance of lactic acid directly influences the mechanical events by inducing a sudden change in the hydrogen-ion concentration at contractile surfaces. If so, there is much interest in the relation of the muscular machinery to the fuel it consumes.

We possess suggestive if not wholly conclusive evidence which indicates that lactic acid is an obligate intermediary in the breakdown of carbohydrate in all animal tissues.

We may therefore suppose that in the evolution of the structure of striated muscle advantage was taken of the existence of this acidic metabolite. Structure and metabolism became mutually adjusted to produce a remarkable and highly efficient machine, in which, at one stage in a cycle, part of the fuel which is to be ultimately burnt becomes temporarily part of the machinery—at this stage it is concerned with the liberation rather than with the supply of energy. It would seem further that to exert its special influence as an acid the metabolite must at a given moment be produced in much larger amount than what is required for immediate oxidation: the balance is therefore returned to the store.

Intimately connected with these peculiar relations between fuel and machinery are the special time relations of the oxidative process to which full attention has been given in the lecture. Oxidations do not immediately and directly supply the energy of contraction. They rather restore potential. They, as it were, wind up the clock: or if you prefer the analogy, oxidations yield energy as does a dynamo when it charges an accumulator. The store in the accumulator may be what actually runs the machine.

In spite of the simplicity in events suggested by the direct relation between carbohydrate and lactic acid in muscle metabolism there is, even from the standpoint of chemistry alone, much yet to be learnt concerning details. If it does not deal with its carbohydrate quite differently from less specialized tissues, we must look somewhere amid the dynamical events, which in muscle connect lactic acid with glycogen or hexose phosphate, for the influence of that catalyst which controls equilibrium between lactic acid and methyl-glyoxal. The discovery of this catalyst by Dakin and its study by Dakin and Dudley constitute one of the most significant contributions ever made to the chemistry of the tissues. Until we know where in the sequence of events the activity of this cell catalyst finds a place, we must in my opinion be content with an incomplete picture of chemical dynamics in the cell.

Mention of glyoxalase leads me to consider very briefly another fundamental matter upon which the study of excised muscle has a bearing. Dakin and Dudley showed, as you will remember, that nearly all functionally active tissues contain this enzyme, so that extracts made from them rapidly convert methyl-glyoxal into lactic acid. The pancreas stands out as an exception. Not only is it probably free from glyoxalase but it contains a factor which inhibits the action of that enzyme. Pancreatic extracts can prevent the formation of lactic acid from methyl-glyoxal by other tissues. Now Winfield and I have shown that pancreatic extracts diminish the velocity of lactic acid formation in chopped muscle, the source of the latter being, as Meyerhof has proved, undoubtedly the carbohydrate of the tissue. The facts suggest that methyl-glyoxal and lactic acid are both intermediate products in the breakdown of carbohydrate and that the pancreas may control the velocity of this breakdown at a stage between the appearance of methyl-glyoxal and its conversion into the acid.

It is not yet clear how we are to relate these possibilities to the occurrence of glycosuria; but it would be wrong, I think, for those who are concerned with diabetes to ignore them.

We shall not understand the whole of the fascinating chemical system which has occupied our attention if we think only of its energy exchanges and the material events which support them.

All the dynamic phenomena we have considered, though they may be displayed to perfection for long periods by muscles excised from the body, are of course displayed only while a certain equilibrium is maintained among a complicated set of factors; the equilibrium which is associated with, and necessary for, the property of irritability.

So long as this is maintained, or, if lost, is capable of restoration, so long are we justified in attributing life to the tissue. A moment must come when the necessary equilibrium is no longer maintained, and this moment is usually—though, as we are to see, not always—associated with, and marked by, a happening which seems to have a more or less critical character—the rigor of death. This may arise as the final result of oxygen lack alone; but we have learnt to make no sharp distinction between the onset of rigor mortis and that gradual progress of fatigue which in active muscle may lead up to it: a progress which is accelerated by deprivation of oxygen and delayed by a proper supply of that essential factor.

It is the accumulation of products of change and not the exhaustion of supplies of oxidisable material which leads to fatigue and ultimately to death in rigor. Fletcher's early work and the work we did together, as well as such observations as those of Joteyko, left little doubt that the prime if not the sole cause of fatigue, and, no less, of death in rigor, is the accumulation of lactic acid. Both phenomena are due to the effect of this upon the colloid machinery of the muscle. Fatigue increases as the accumulation increases and the critical moment of rigor mortis (in so far as it is critical) corresponds with the attainment of a certain concentration of acid.

These are the common and obvious events. When they occur they overshadow other failures in equilibrium which may nevertheless be as fatal to what we call the life of the tissue. An excised amphibian muscle, when quiescent in an atmosphere of oxygen, does not accumulate lactic acid, and never displays rigor mortis at all. Yet though it lives surprisingly long, it is certainly not immortal. It ultimately ceases to be irritable and we must then speak of its death; but it is death without rigor. What now is the cause of death? Miss D. L. Foster and Miss D. M. Moyle have carried out in my laboratory some experiments which bear upon the answer to this question and the results seem to be of great interest.

If frog's limb muscles are kept in oxygen at 0° C. they remain irritable for periods which vary with the season from about a fortnight to three weeks. The irritability declines relatively fast immediately after the first few days, then remains constant for a period, and later declines more rapidly to zero. But the muscles when, finally, they fail entirely to respond to the strongest electrical stimulus show no signs of rigor mortis. In their flaccidity and in their general appearance they resemble perfectly fresh muscles. Corresponding with this they are found to contain only that original "resting minimum" of lactic acid which must have been present when they were originally removed from the frog. In parenthesis it may be stated that this failure to accumulate lactic acid is not due to the complete inhibition by the low temperature of the chemical processes concerned. In nitrogen the muscles at 0° C. accumulate lactic acid at a steady rate.

What, however, is noteworthy in the condition of muscles which have lost excitability during long exposure in oxygen at 0° C. is the fact that their chemical mechanisms remain intact. Placed in air at 40° C. they rapidly come to exhibit the normal maximum of lactic acid, and they show no less the usual acid production which results from injury, or, say, the action of chloroform vapor. Although they transmit no change of electric potential, they show, in further proof of their chemical integrity, a demarcation current when injured. Judged by chemical criteria they are alive, while tested by electric stimulation they appear to be dead. There is, as it were, a dislocation between stimulus and energy discharge. A narcotized muscle may be said to show a similar dislocation: but the underlying conditions are in this case different; for narcosis encourages the accumulation of lactic acid. What then is the state of affairs in the inexcitable muscles we have been considering? One hypothesis suggests itself. Nernst's theory postulates the free movement of ions towards a critical concentration at a surface as an essential happening in the excitation of a tissue. Now in systems containing both colloids and electrolytes in solution there is a tendency with progress of time for the associations between them to assume increased stability. This may occur in muscles which remain for long periods at 0° C., thus ultimately the concentration of ions which are free to move may become too small to form the necessary assemblage at a surface. Some confirmation of such a view is to be found in the fact that, though the muscles in question have suffered no loss of material save a small fraction of their glycogen, the osmotic pressure in their fibres is much less than that which they exhibit when first removed from the animal.

NOTES ON NEW BOOKS

A Diabetic Manual for the Use of Doctor and Patient. By ELLIOTT P. JOSLIN. 2d ed. (*Philadelphia and New York, Lea and Febiger, 1919.*)

The second edition of this little book is an indication of its continued usefulness. Here in a few pages, the writer in his forceful epigrammatic way has summarized most that is of practical value in the management of the diabetic. Throughout there is a background of personal experience which lends weight to the dicta expressed in the text. A study of this book will keep the physician, who does not specialize in diabetes, on the right track when called on to handle the occasional case, and the patient himself can glean from it sound counsel without becoming over-educated.

A. L. B.

Diabetes, a Handbook for Physicians and Their Patients. By PHILIP HOROWITZ. Cloth, $2.00. (*New York, Paul B. Hoeber, 1920.*)

This little book contains a useful summary of the author's methods of handling diabetics. The subject is well presented, but adds little to previous works of similar character.

A. L. B.

The Oxford Medicine. By Various Authors. Edited by H. A. CHRISTIAN and J. MACKENZIE. (*New York, Oxford University Press.*)

This volume keeps up the general excellent standard of its predecessors. The subjects, including diseases of the gastro-intestinal tract, liver, pancreas, and peritoneum are adequately treated. The articles on nephritis are of outstanding value. Here for the first time in a text-book do we find a modern consideration of the subject based on the facts brought out by recent chemical and clinical studies. The writer has wisely taken up the subject from the clinical standpoint, and avoided the confusion of the combined etiological, pathological, clinical classification which we usually find.

A. L. B.

Heart Affections. Their Recognition and Treatment. By S. C. SMITH. Cloth, $5.00. (*F. A. Davis Company, Philadelphia, 1920.*)

In this little compendium the writer has adopted a rather new method of exposition. Without going at great length into experimental fundamentals, he brings the more modern methods of cardiac study en rapport with pure clinical observation. While hardly complete enough to serve as a treatise for the beginner, the book seems to us most valuable to the practitioner who is accustomed to the point of view of fifteen years ago and desires to add to his armamentarium the newer physical methods of study. The material is clearly presented and while the pure cardiologist might take exception to small points, perhaps more on account of their incompleteness and lack of qualification than for other reasons, the book seems to us to have accomplished well the author's purpose.

A. L. B.

The Treatment of Acute Infectious Diseases. By F. S. MEARA. Second Edition. (*MacMillan Company, New York, 1921.*)

Text-books on treatment are in the main unsatisfactory; either they are brief, inadequate outlines, or they catalogue at length a variety of possible measures without due criticism. It is a delight therefore to read Meara's admirable treatise, and to find there detailed practical directions as to just what to do, based on sound scientific principles, but tempered by the author's extensive experience. The introductory sections on fever and on diet in infectious disease strike fundamental keynotes which recur in the succeeding sections. As to the actual substance of the book the reviewer finds himself in complete agreement with the author's ideas and methods. The detailed method of exposition, especially with reference to local measures and modes of drug administration, is highly satisfactory. A good deal is said about vaccine therapy, but it is only fair to say that the writer makes no undue claims. Finally, one of the most pleasing features of the book is its readable style which allows one to peruse its successive pages without weariness.

A. L. B.

The Principles of Immunology. By H. T. KARSNER and E. E. ECKER. Cloth $5.00. (*J. B. Lippincott Company, Philadelphia and London, 1921.*)

This book represents an up-to-date summary of its subject. As stated by the authors, previous works have been freely drawn on and many sections are reminiscent of other compendiums. The work seems to be of distinct value, combining as it does theory with practical conclusions and confining itself to that which has been pretty clearly established as fact.

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